United States
Department of
Agriculture

Agricultural
Research
Service

Miscellaneous
Publication
Number 1434

# Standard Tests to Characterize Pest Resistance in Alfalfa Cultivars

# Abstract

U.S. Department of Agriculture, Agricultural Research Service. 1984. Standard Tests to Characterize Pest Resistance in Alfalfa Cultivars. U.S. Department of Agriculture Miscellaneous Publication No. 1434, 44 p.

This publication contains information for use as a working document of pest resistance research in alfalfa. It is intended for personnel concerned with breeding for alfalfa-pest resistance, forage and seed production, and extension or marketing. Included are a listing of the disease, insect, and nematode pests that substantially limit growth and production of alfalfa; U.S. distribution and severity maps for the most important pests; scientists with expertise on specific pests; and established resistance evaluation procedures for 20 of the major pests.

Keywords: alfalfa, cultivars, screening, insects, pests, resistance, disease, distribution and severity maps

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Agricultural Research Service
in cooperation with
Agricultural Experiment Stations
in
Arizona, California, Kansas, Minnesota,
Nebraska, Nevada, Utah, and Washington

This publication supersedes and enlarges ARS-NC-19, "Standard Tests To Characterize Pest Resistance in Alfalfa Varieties," issued September 1974.

Issued June 1984

## Preface

This publication compiles current host-plant evaluation information for the major alfalfa pests in North America. The information was assembled by the Alfalfa Improvement Conference Committee on "Standard Tests for Characterizing Disease and Insect Resistance of Alfalfa Cultivars." The first edition (ARS-NC-19, 1974) was approved by the 23d Alfalfa Improvement Conference in 1972. Members attending the 26th Alfalfa Improvement Conference at Brookings, S. Dak., June 6–8, 1978, requested that a revised edition be developed.

The committee was assigned to (1) review the available information on evaluating pest resistance in alfalfa cultivars and determine whether information gaps exist, (2) identify and describe pest-evaluation procedures that could be considered as standard tests, (3) identify persons and locations that can provide information about cultivar-evaluation tests for specific types of pest resistance, and (4) collect seed of check cultivars for distribution to scientists conducting standard pest-evaluation tests.

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# Standard Tests To Characterize Pest Resistance in Alfalfa Cultivars

## Need to Standardize Pest-Resistance Evaluation

Alfalfa (*Medicago sativa* L.) ranks fourth in the United States in acreage (26,394,000) and dollar value ($5.6 billion) among all agricultural crops (Agricultural Statistics, 1982). Alfalfa is produced in all 50 States. Alfalfa's extremely wide area of adaptation and its perennial growth habit cause it to be exposed to a large number of disease, insect, and nematode pests. In 1970, alfalfa scientists estimated that the combined annual losses from all pests amounted to about 40 percent of the national production.

The importance of pest resistance was first demonstrated in 1942 with the release of two bacterial wilt-resistant cultivars—'Ranger' for the northern Great Plains area and 'Buffalo' for the central Plains area. Since then, cultivars have been released that have resistance to anthracnose, alfalfa weevil, blue alfalfa aphid, verticillium wilt, fusarium wilt, pea aphid, phytophthora root rot, spotted alfalfa aphid, and stem nematode. Research techniques are being developed for selecting resistance to other important pests.

The development of pest-resistant alfalfa cultivars has been an important contribution to agriculture. Most alfalfa acreage in the United States now is planted with cultivars that were selected for resistance to one or more pests. During the past decade, alfalfa acreage in the United States has remained constant, whereas yields have increased nearly 20 percent. Much of this increase can be credited to cultivars with increased types and levels of pest resistance. Some types of pest resistance also have increased forage quality.

A primary goal of most alfalfa-breeding programs is to develop new cultivars with multiple pest resistance. There are many pests, however, and cultivar development takes time. Alfalfa breeders, plant pathologists, and entomologists must assign priorities to each type of resistance. They also must use the most effective methods for selecting and evaluating resistant germplasm. Growers, extension personnel, marketing specialists, and research scientists need uniform procedures to describe levels of pest resistance of cultivars. The Plant Variety Protection Act of 1970 also increased the need for standardizing the characterization of pest resistance. This publication presents a summary of pest resistance information to help the alfalfa industry develop and describe pest-resistant cultivars.

## Characterizing Disease Resistance

### Background

Plant disease may be caused by either abiotic agents or biotic agents. Abiotic agents include nutrient deficiencies, toxicities, physiogenic spotting, and air pollution damage. Differences occur among cultivars in their reaction to some abiotic agents. The selection of strains resistant to air pollution by Howell and others *(42)*[1] is an example. In general, however, little progress has been made in developing resistance to abiotic agents. This publication, therefore, is confined to diseases caused by biotic agents.

Biotic agents that incite diseases in alfalfa are bacteria, fungi, viruses, and mycoplasmas. Graham and others *(29)* reported that at least 70 different pathogens occur in alfalfa and that about 30 limit the growth and production of the crop. A survey of the United States by the Alfalfa Improvement Conference Committee identified 22 diseases that were problems in more than one State. Of these diseases, 19 were identified as problems in enough States so that national distribution and severity maps could be developed.

Some research information is available on nearly every important alfalfa disease in the United States *(27,46)*, but often only one scientist may have worked with a particular disease. Even though information is available and variation in host-plant resistance has been reported for most important diseases, reliable evaluation procedures are available for only bacterial wilt, anthracnose, common leaf spot, downy mildew, fusarium wilt, lepto leaf spot, phytophthora root rot, rust, and verticillium wilt.

Sufficient information is available to screen for resistance to bacterial leaf spot, rhizoctonia crown rot, sclerotinia crown and stem rot, spring black stem, stemphylium leaf spot, summer black stem, yellow leaf blotch, and alfalfa mosaic virus. Unfortunately, the procedures used to determine resistance to these diseases usually rely on natural field epiphytotics; therefore, reliable field evaluation procedures are available only on occasion. With additional research, it may be possible to develop evaluation procedures for most, if not all, important diseases in alfalfa.

Reliable evaluation procedures require that uniform infections be repeated in subsequent tests. The percentage of resistant plants and average disease severity index (ASI)

[1] Italic numbers in parentheses refer to Literature Cited, p. 33.

should both be used to describe cultivar reaction to diseases. Cultivar evaluations should be based on individual plant scores rather than on solid-seeded plots. A 1 to 5 rating when feasible (1 = no disease, 5 = severely diseased) is favored. Ratings for each pathogen should be based on specific lesion types or degree of infection (some diseases may require fewer or more than five classes). Rating values should be consistent from test to test. Generally, at least 100 plants should be used for evaluating a cultivar, and resistant and susceptible check cultivars should be included in every test. The susceptible check cultivar can be used to estimate percentage of escapes in a test. The cultivar being evaluated should be compared to the resistant check. Standard seed lots of the check cultivars (when available) should be used. Although no agreement was reached on the number of tests required for evaluation, individual scientists should base their decision on the number of escapes and relative precision of their data.

In describing the relative disease resistance of alfalfa cultivars, the word "tolerant" should be used to denote a type of host pathogen interaction but should not be used to denote low levels of disease resistance. The term "immune" probably will have no place in alfalfa cultivar descriptions because of the difficulty with reaching homozygosity for any trait.

### Summary of Resistance Information by Disease

Distribution and severity maps for 19 important diseases in the United States appear on pages 14 to 19. These maps are based on observations and opinions of research and extension specialists from nearly every State. Scientists with expertise on specific diseases or pathogens are identified as resource people who can supply additional information about developing and evaluating specific types of disease resistance in alfalfa. Check cultivars are identified for disease evaluations where sufficient information was available.[2] In nearly all instances, public cultivars are designated as checks instead of proprietory cultivars. This designation ensures the availability of seed after the cultivars are no longer commercially available. We attempted to keep the number of check cultivars to a minimum. For these reasons, some cultivars with higher levels of resistance and more extensive distribution may not have been selected as checks. The availability and type of screening and evaluation procedures (L = laboratory, F = field tests) also are indicated.

**Tabulation summarizing available resistance information by disease**

**Bacterial Leaf Spot**
*Xanthomonas alfalfae*
(Riker, Jones & Davis) Dows.

Distribution and severity map: Figure 1
Scientists and locations with expertise:[3] 31 and 32

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | KS 76 | — |
| Nondormant types: | — | — |

Screening procedures: L
Evaluation procedures: None

**Bacterial Wilt**
*Corynebacterium insidiosum*
(McCull) H. L. Jens.

Distribution and severity map: Figure 2
Scientists and locations with expertise: 1, 8, 16, 19, and 35

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Vernal | Narragansett |
| Nondormant types: | — | Sonora |

Screening procedures: L and F
Evaluation procedures: F (p. 20)

[2]See appendix, p. 37, for approximate levels of pest resistance expected for check cultivars.
[3]See listing of resource scientists on p. 12.

**Anthracnose**
*Colletotrichum trifolii*
Bain

Distribution and severity map: Figure 3
Scientists and locations with expertise: 6, 9, 19, 24, and 37

| Check cultivars: | | Resistant | Susceptible |
|---|---|---|---|
| Dormant and semidormant types: | Race 1: | Arc, Saranac AR | Saranac |
| | Race 2: | Saranac AR | Saranac, Arc |
| Nondormant types: | | — | — |

Screening procedures: L
Evaluation procedures: L (p. 21)

**Common Leaf Spot**
*Pseudopeziza medicaginis*
(Lib.) Sacc.

Distribution and severity map: Figure 4
Scientists and locations with expertise: 8, 17, and 35

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | MSA-CW3An3, Ramsey | Ranger |
| Nondormant types: | — | Moapa 69 |

Screening procedures: L and F
Evaluation procedures: L and F (p. 21)

**Downy Mildew**
*Peronospora trifoliorum*
d By.

Distribution and severity map: Figure 5
Scientists and locations with expertise: 28, 31, and 32

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Saranac | Kanza |
| Nondormant types: | — | — |

Screening procedures: L and F
Evaluation procedures: L and F (p. 23)

**Fusarium Wilt**
*Fusarium oxysporum*
Schlecht f. sp. *medicaginis*
(Weimer) Snyd. & Hans.

Distribution and severity map: Figure 6
Scientists and locations with expertise: 1, 6, 7, 8, 24, 25, and 37

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Agate | Narragansett |
| Nondormant types: | Moapa 69 | — |

Screening procedures: L and F
Evaluation procedures: L and F (p. 24)

**Fusarium Root and Crown Rot**
*Fusarium solani*
(Mart.) Appel & Wr.,
*Fusarium roseum*
Lk. ex Fr. emend.
Snyd. & Hans.

Distribution and severity map: Figure 7
Scientists and locations with expertise: 17 and 24

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: None
Evaluation procedures: None

**Lepto Leaf Spot**
*Leptosphaerulina briosiana*
(Poll.) Graham & Luttrell

Distribution and severity map: Figure 8
Scientists and locations with expertise: 8, 13, and 17

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | MSA-PL-L | Ranger |
| Nondormant types: | — | Moapa 69 |

Screening procedures: L and F
Evaluation procedures: L (p. 22)

**Phytophthora Root Rot**
*Phytophthora megasperma*
Drechs. f. sp. *medicaginis*

Distribution and severity map: Figure 9
Scientists and locations with expertise: 1, 7, 8, 9, 14, 21, 35, and 37

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Agate | Saranac |
| Nondormant types: | CUF 101, MnP-D1 | Sonora |

Screening procedures: L and F
Evaluation procedures: L and F (p. 25)

**Phymatotrichum Root Rot**
*Phymatotrichum omnivorum*
(Shear) Dug.

Distribution and severity map: Figure 10
Scientists and locations with expertise: 7, 14, and 28

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: None
Evaluation procedures: None

**Rhizoctonia Crown Rot and Root Canker**
*Rhizoctonia solani*
Kuehn

Distribution and severity map: Figure 11
Scientists and locations with expertise: 7, 8, and 18

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: None
Evaluation procedures: None

**Rust**
*Uromyces striatus*
Schroet.

Distribution and severity map: Figure 12
Scientists and locations with expertise: 6, 13, 17, and 30

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | MSA-CW3An3 | Ranger, Saranac |
| Nondormant types: | — | Moapa 69 |

Screening procedures: L and F
Evaluation procedures: L (p. 26)

**Sclerotinia Crown and Stem Rot**
*Sclerotinia trifoliorum* Eriks.

Distribution and severity map: Figure 13
Scientists and locations with expertise: 6 and 37

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: L and F
Evaluation procedures: None

**Spring Black Stem**
*Phoma medicaginis* Malbr. & Roum.
*(Ascochyta imperfecta)*

Distribution and severity map: Figure 14
Scientists and locations with expertise: 8, 9, and 17

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Ramsey | Lahontan, Ranger |
| Nondormant types: | — | — |

Screening procedures: L and F
Evaluation procedures: May be possible on occasion in field.

**Stagonospora Crown and Root Rot and Leaf Spot**
*Stagonospora meliloti* (Lasch) Petr.

Distribution and severity map: None
Scientists and locations with expertise: 7, 9, and 34

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: None
Evaluation procedures: None

**Stemphylium Leaf Spot**
*Pleospora herbarum* (Pers. ex. Fr.) Rab.
(*Stemphylium botryosum* Wallr.)

Distribution and severity map: Figure 15
Scientists and locations with expertise: 9, 17, and 36

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: F
Evaluation procedures: May be possible on occasion in field.

**Summer Black Stem**
*Cercospora medicaginis* Ell. & Ev.

Distribution and severity map: Figure 16
Scientists and locations with expertise: 31 and 32

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | Lahontan |
| Nondormant types: | — | — |

Screening procedures: F
Evaluation procedures: May be possible on occasion in field.

**Verticillium Wilt**
*Verticillium albo-atrum*
Reinke & Berth.

Distribution and severity map: Figure 17
Scientists and locations with expertise: 4 and 25

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Vertus | Vernal, Saranac |
| Nondormant types: | — | Sonora |

Screening procedures: L
Evaluation procedures: L (p. 26)

**Yellow Leaf Blotch**
*Leptotrochila medicaginis*
(Fckl.) Schuepp.

Distribution and severity map: Figure 18
Scientists and locations with expertise: 27

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Teton | Ranger |
| Nondormant types: | — | — |

Screening procedures: L
Evaluation procedures: L (p. 27)

**Alfalfa Dwarf**
(Rickettsia-like bacterium)

Distribution and severity map: None
Scientists and locations with expertise: 7

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: None
Evaluation procedures: None

**Alfalfa Mosaic Virus**

Distribution and severity map: Figure 19
Scientists and locations with expertise: 8 and 10

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: L
Evaluation procedures: None

**Witches'-Broom**
(mycoplasma)

Distribution and severity map: None
Scientists and locations with expertise: 7

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: None
Evaluation procedures: None

## Characterizing Insect Resistance

### Background

More than 100 insect species have been recorded as injurious to alfalfa, according to App and Manglitz *(1)*. In a survey of the United States, however, only 11 alfalfa insects were identified by research and extension specialists as serious over large areas. Additional insects were identified as local problems. This publication is restricted to the 11 widely distributed insect pests.

Insect pests described here injure the alfalfa crop in several ways. Some reduce plant yields and stands by consuming portions of the plant or by sucking plant juice. Others influence yield and quality by injecting toxins into the plant that cause stunting and distorted growth. Some insect pests attack the flowering parts or developing seed and thus reduce seed yields. Insects also can serve as vectors of plant viruses and mycoplasmas or provide avenues of entrance for disease organisms. Insect mobility and the many types of feeding injuries they produce often make breeding for insect resistance more difficult than breeding for some types of disease resistance. The recent identification of biotypés for some alfalfa insects further complicates breeding for insect resistance. This is especially true for the pea aphid and spotted alfalfa aphid.

Considering the complexities of insect, host plant, and environmental interactions, it is not surprising that reliable evaluation procedures are available for only the alfalfa seed chalcid, alfalfa weevil, Egyptian alfalfa weevil, potato leafhopper, pea aphid, blue alfalfa aphid, and spotted alfalfa aphid. Unfortunately, only the pea aphid, blue alfalfa aphid, spotted alfalfa aphid, and alfalfa seed chalcid tests can be conducted in the laboratory or greenhouse. Most insect screening and evaluation procedures depend on natural field infestations of the target insect, which often creates uncertainties about levels and uniformity of infestations among test years. Field evaluations also create the problem that damage by the target insect may be confounded with disease injury and with injury caused by other insects.

Although more difficulties appear to arise with insect resistance evaluations than with disease evaluations, the same principles should apply in evaluating cultivar resistance to both types of pests. Dependable results require that relatively uniform insect infestation levels be developed and repeated in subsequent tests. A desirable practice is to check periodically the identification of the test insect species because similar-appearing species may confound the results. Whenever possible, cultivar evaluations should be based on scoring individual plants rather than solid-seeded plots. A numerical-rating system based on specific levels of damage or on insect population counts per plant should be used, and rating values should be consistent from one test to the next. The percentage of resistant plants, ASI, or average population counts per plant should be used to compare cultivars statistically with standard seed lots of resistant and susceptible check cultivars.

Insect host-plant resistance often can be expressed using such specific terms as "nonpreference," "tolerance," and "antibiosis" *(72)*. Although this information may be difficult to obtain, specific terminology should be used, when possible, in describing cultivar evaluations. This practice will promote a better understanding of insect resistance and may be useful in planning pest-management programs.

### Summary of Resistance Information by Insect Species

Distribution and severity maps for eight of the most widely spread insect species in the United States appear on pages 14 to 19. Scientists who can serve as resource people for problems concerned with developing host-plant resistance are identified. Also, check cultivars have been identified where sufficient information is available[2] and the availability and type of screening and evaluation procedures (L = laboratory, F = field tests) are indicated.

**Tabulation summarizing available resistance information by insect species**

**Alfalfa Plant Bug**
*Adelphocoris lineolatus* (Goeze)

Distribution and severity map: None
Scientists and locations with expertise:[3] 1, 16, and 20

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: F
Evaluation procedures: None

**Alfalfa Seed Chalcid**
*Bruchophagus roddi* (Gussakovsky)

Distribution and severity map: None
Scientists and locations with expertise: 22, 23, 28, and 39

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | Ranger |
| Nondormant types: | — | Sonora |

Screening procedures: F and L
Evaluation procedures: F and L (p. 27)

**Alfalfa Weevil**
*Hypera postica* (Gyllenhal)

Distribution and severity map: Figure 20
Scientists and locations with expertise: 3, 5, 6, 15, 16, 20, 26, 29, 31, and 33

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Team, Arc | Ranger, Saranac |
| Nondormant types: | — | — |

Screening procedures: F and L
Evaluation procedures: F (p. 28)

**Egyptian Alfalfa Weevil**
*Hypera brunneipennis* (Boheman)

Distribution and severity map: Figure 21
Scientists and locations with expertise: 18, 23, 28, and 33

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: F
Evaluation procedures: F (p. 28) (see procedures for alfalfa weevil)

[2]See appendix, p. 37, for approximate level of pest resistance expected for the check cultivars.
[3]See listing of resource scientists on p. 12.

**Lygus spp.**

Distribution and severity map: None
Scientists and locations with expertise: 16, 20, 22, 23, 28, and 39

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | Ranger |
| Nondormant types: | — | Moapa 69 |

Screening procedures: F
Evaluation procedures: None

**Meadow Spittlebug**
*Philaenus spumarius*
(Linnaeus)

Distribution and severity map: Figure 22
Scientists and locations with expertise: 29 and 38

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Culver | Ranger |
| Nondormant types: | — | — |

Screening procedures: L
Evaluation procedures: None

**Pea Aphid**
*Acyrthosiphon pisum*
(Harris)

Distribution and severity map: Figure 23
Scientists and locations with expertise: 12, 15, 16, 18, 20, 22, 23, 31, and 39

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Kanza, Baker | Ranger, Vernal |
| Nondormant types: | PA-1, CUF 101 | Caliverde, Moapa 69 |

Screening procedures: L
Evaluation procedures: L (p. 28)

**Blue Alfalfa Aphid**
*Acyrthosiphon kondoi*
Shinji

Distribution and severity map: Figure 24
Scientists and locations with expertise: 12, 15, 18, 23, 28, 31, and 33

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | CUF 101 | PA-1, Caliverde |

Screening procedures: F and L
Evaluation procedures: F and L (p. 29)

**Spotted Alfalfa Aphid**
*Therioaphis maculata*
(Buckton)

Distribution and severity map: Figure 25
Scientists and locations with expertise: 12, 15, 16, 18, 20, 22, 23, 28, 31, and 39

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Kanza, Baker | Ranger, Team |
| Nondormant types: | Mesa-Sirsa, CUF 101 | Caliverde |

Screening procedures: L
Evaluation procedures: L (p. 29)

**Potato Leafhopper**
*Empoasca fabae*
(Harris)

Distribution and severity map: Figure 26
Scientists and locations with expertise: 2, 5, 6, 13, 15, 16, 20, 26, 29, 31, and 38

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | MSA-CW3An3 | Ranger |
| Nondormant types: | — | — |

Screening procedures: F
Evaluation procedures: F (p. 29)

*Empoasca mexara*
Ross and Moore

Distribution and severity map: Figure 26
Scientists and locations with expertise: 23 and 28

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | — |
| Nondormant types: | — | — |

Screening procedures: None
Evaluation procedures: None

## Background

Not much is known about the damage caused by many nematodes associated with alfalfa according to Graham and others *(29)*. This lack of information is due in part to the lack of research on alfalfa nematodes and the lack of distinctive aboveground symptoms caused by nematode injury. Presently, the stem nematode and two species of root-knot nematode are the primary parasitic nematode species known on alfalfa. Resistant cultivars or germplasm have been developed to all three species.

## Summary of Resistance Information by Nematode Species

Distribution and severity maps for the three primary parasitic nematode species on alfalfa in the United States appear on page 19. Scientists who can serve as resource people for problems concerned with developing nematode-resistant cultivars are identified. Check cultivars and standardized-screening procedures have been established for the three types of nematode resistance.[2] Availability and type of screening and evaluation procedures (L = laboratory, F = field tests) are indicated also.

**Tabulation summarizing available resistance information by nematode species**

**Stem Nematode**
*Ditylenchus dipsaci*
(Kuhn) Filipjev

Distribution and severity map: Figure 27
Scientists and locations with expertise:[3] 6, 11, 12, 25, 28, and 35

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Lahontan | Ranger |
| Nondormant types: | Caliverde 65 | Moapa 69 |

Screening procedures: L
Evaluation procedures: L (p. 30)

**Northern Root-Knot Nematode**
*Meloidogyne hapla*
Chitwood

Distribution and severity map: Figure 28
Scientists and locations with expertise: 6, 11, 12, 25, and 34

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | Nev. Syn. XX | Lahontan |
| Nondormant types: | Nev. Syn. YY | Mesa-Sirsa |

Screening procedures: L
Evaluation procedures: L (p. 30)

**Southern Root-Knot Nematode**
*Meloidogyne incognita*
Chitwood and
*Meloidogyne javanica*
(Treub) Chitwood

Distribution and severity map: Figure 28
Scientists and locations with expertise: 6, 11, 12, 25, and 34

| Check cultivars: | Resistant | Susceptible |
|---|---|---|
| Dormant and semidormant types: | — | Lahontan |
| Nondormant types: | Moapa 69 | Caliverde 65 |

Screening procedures: L
Evaluation procedures: L (p. 30)

[2]See appendix, p. 37, for approximate levels of pest resistance expected for the check cultivars.
[3]See listing of resource scientists on p. 12.

## Resource Scientists

The following list includes scientists familiar with various phases of alfalfa pest resistance research from State agricultural experiment stations and the U.S. Department of Agriculture (USDA). It is intended to be, primarily, a list of resource personnel who are knowledgeable about pest resistance evaluation procedures.

1. D. K. Barnes, USDA-ARS, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minn. 55108.

2. R. A. Byers, USDA-ARS, U.S. Regional Pasture Research Laboratory, University Park, Pa. 16802.

3. W. V. Campbell, Department of Entomology, North Carolina State University, Raleigh, N.C. 27650.

4. A. Christen, Irrigated Agriculture Research and Extension Center, Prosser, Wash. 99350.

5. T. C. Elden, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, Md. 20705.

6. J. H. Elgin, Jr., USAD-ARS, Beltsville Agricultural Research Center, Beltsville, Md. 20705.

7. D. C. Erwin, Plant Pathology Department, University of California, Riverside, Calif. 92501.

8. F. I. Frosheiser, USDA-ARS, Department of Plant Pathology, University of Minnesota, St. Paul, Minn. 55108.

9. D. G. Gilchrist, Department of Plant Pathology, University of California, Davis, Calif. 95616.

10. A. H. Gold, Department of Plant Pathology, University of California, Berkeley, Calif. 94720.

11. G. D. Griffin, USDA-ARS, Department of Plant Science, Utah State University, Logan, Utah 84322.

12. B. J. Hartman, USDA-ARS, University of Nevada, Reno, Nev. 89557.

13. R. R. Hill, Jr., USDA-ARS, U.S. Regional Pasture Research Laboratory, University Park, Pa. 16802.

14. R. B. Hine, Department of Plant Pathology, University of Arizona, Tucson, Ariz. 85721.

15. E. Horber, Entomology Department, Kansas State University, Manhattan, Kans. 66506.

16. W. R. Kehr, USDA-ARS, 333 Keim Hall, University of Nebraska, Lincoln, Nebr. 68583.

17. K. T. Leath, USDA-ARS, U.S. Regional Pasture Research Laboratory, University Park, Pa. 16802.

18. W. F. Lehman, University of California, 1004 E. Holton Road, El Centro, Calif. 92243.

19. F. L. Lukezic, Department of Plant Pathology, Pennsylvania State University, University Park, Pa. 16802.

20. G. R. Manglitz, USDA-ARS, Department of Entomology, University of Nebraska, Lincoln, Nebr. 68583.

21. D. P. Maxwell, Department of Plant Pathology, University of Wisconsin, Madison, Wis. 53706.

22. B. A. Melton, Department of Agronomy, New Mexico State University, Las Cruces, N. Mex. 88003.

23. M. W. Nielson, USDA-ARS, Forage Insects Research Laboratory, 2000 East Allen Road, Tucson, Ariz. 85719.

24. S. A. Ostazeski, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, Md. 20705.

25. R. N. Peaden, USDA-ARS, Irrigated Agriculture Research & Extension Center, Prosser, Wash. 99350.

26. R. H. Ratcliffe, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, Md. 20705.

27. M. D. Rumbaugh, Department of Plant Science, Utah State University, Logan, Utah 84322.

28. M. H. Schonhorst, Department of Agronomy, University of Arizona, Tucson, Ariz. 85721.

29. R. E. Shade, Department of Entomology, Purdue University, Lafayette, Ind. 47907.

30. R. T. Sherwood, USDA-ARS, U.S. Regional Pasture Research Laboratory, University Park, Pa. 16802.

31. E. L. Sorensen, USDA-ARS, Department of Agronomy, Kansas State University, Manhattan, Kans. 66506.

32. D. L. Stuteville, Department of Plant Pathology, Kansas State University, Manhattan, Kans. 66506.

33. C. G. Summers, University of California, San Joaquin Valley Agricultural Research and Extension Center, Parlier, Calif. 93648.

34. L. R. Teuber, Department of Agronomy and Range Science, University of California, Davis, Calif. 95616.

35. B. D. Thyr, USDA-ARS, University of Nevada, Reno, Nev. 89507.

36. R. K. Webster, Department of Plant Pathology, University of California, Davis, Calif. 95616.

37. R. E. Welty, USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh, N.C. 27607.

38. M. C. Wilson, Department of Entomology, Purdue University, Lafayette, Ind. 47907.

39. M. L. Wilson, Department of Agronomy, New Mexico State University, Las Cruces, N. Mex. 88003.

## Availability of Check Cultivars

Seed of 21 check cultivars cited in this publication have been assembled and placed in cold storage.[2] These cultivars include:

| | | |
|---|---|---|
| Agate | Kanza | Saranac |
| Arc | Lahontan | Saranac AR |
| Baker | Mesa-Sirsa | Sonora |
| Caliverde | Moapa 69 | Team |
| Caliverde 65 | Narragansett | Teton |
| CUF 101 | Ramsey | Vernal |
| Culver | Ranger | Vertus |

For most cultivars, 4.5 kg of foundation seed were obtained. Breeder, registered, or certified seed had to be substituted for several cultivars. This seed is intended for the exclusive use of scientists conducting pest-resistance evaluation tests. Up to 20 g of seed per cultivar will be supplied to a scientist on request.

Seven germplasm lines are listed also as checks. Because of short supplies of seed, lesser amounts of the germplasm lines can be supplied than for the cultivars.

Request seed of the check cultivars and germplasm lines from J. H. Elgin, Jr., USDA-ARS, Field Crops Laboratory, Building 001, BARC-West, Beltsville, Md. 20705.

[2]See appendix, p. 37, for approximate levels of pest resistance expected for the check cultivars.

## Distribution and Severity of Alfalfa Pests

### Diseases

- Not known to occur or occurs but is not an important problem
- Occasionally causes significant losses on susceptible cultivars
- Frequently causes significant losses on susceptible cultivars

Figure 1.—Bacterial leaf spot, *Xanthomonas alfalfae* (Riker, Jones & Davis) Dows.

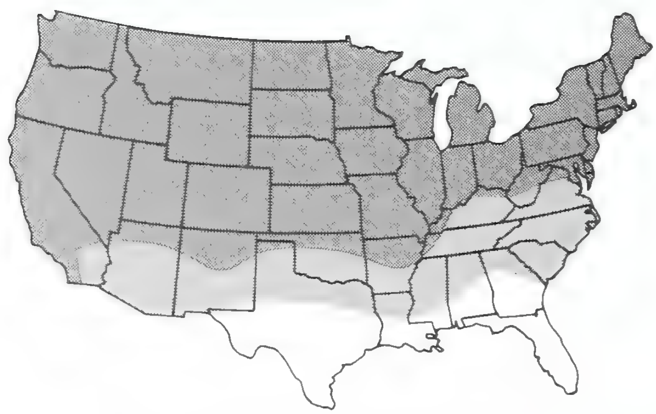


Figure 2.—Bacterial wilt, *Corynebacterium insidiosum* (McCull.) H. L. Jens.

Figure 3.—Anthracnose, *Colletotrichum trifolii* Bain.

Figure 4.—Common leaf spot, *Pseudopeziza medicaginis* (Lib.) Sacc.

Figure 5.—Downy mildew, *Peronospora trifoliorum* d By.

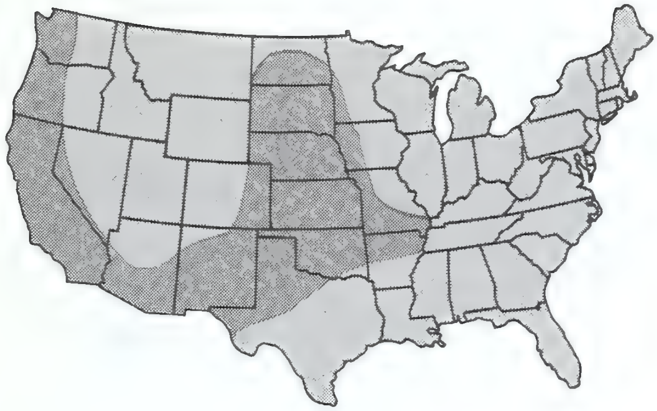
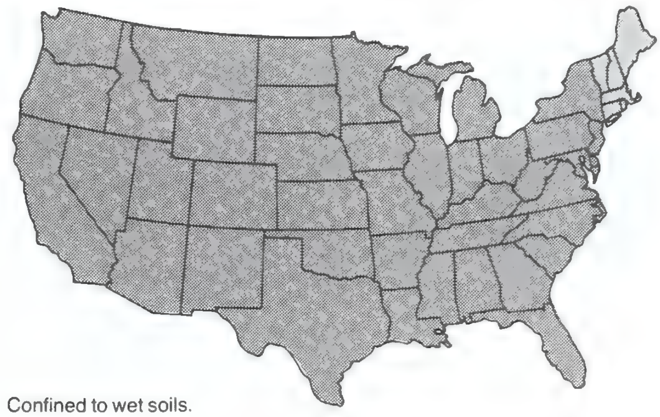


Figure 6.—Fusarium wilt, *Fusarium oxysporum* Schlecht f. sp. *medicaginis* (Weimer) Snyd. & Hans.

Figure 7.—Fusarium root and crown rot, *Fusarium solani* (Mart.) Appel & Wr., *F. roseum* LK.

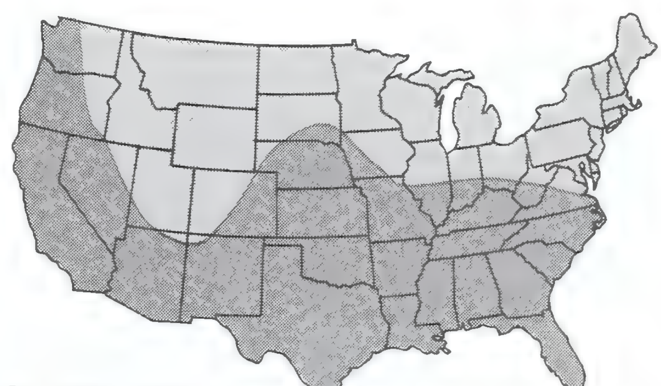
Figure 8.—Lepto leaf spot, *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell.



Confined to wet soils.

Figure 9.—Phytophthora root rot, *Phytophthora megasperma* Drechs. f. sp. *medicaginis*.

Figure 10.—Phymatotrichum root rot, *Phymatotrichum omnivorum* (Shear) Dug.



Root canker primarily in Calif., Nev., Ariz., N. Mex.

Figure 11.—Rhizoctonia crown rot and root canker, *Rhizoctonia solani* Kuehn.

### Diseases (continued)

- Not known to occur or occurs but is not an important problem
- Occasionally causes significant losses on susceptible cultivars
- Frequently causes significant losses on susceptible cultivars
- Figure 19 only: Usually present and causes losses on susceptible cultivars

Figure 12.—Alfalfa rust, *Uromyces striatus* Schroet.

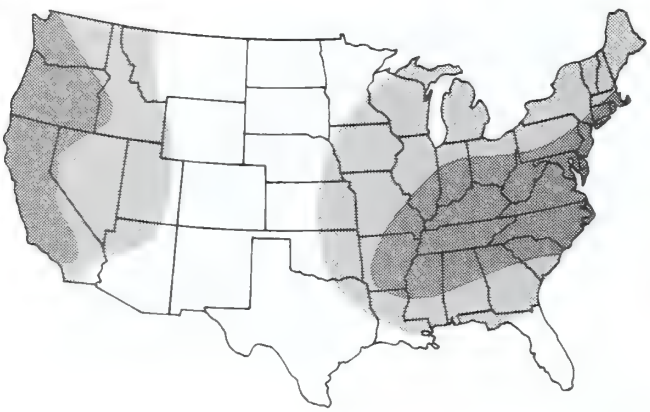


Figure 13.—Sclerotinia crown and stem rot, *Sclerotinia trifoliorum* Eriks.

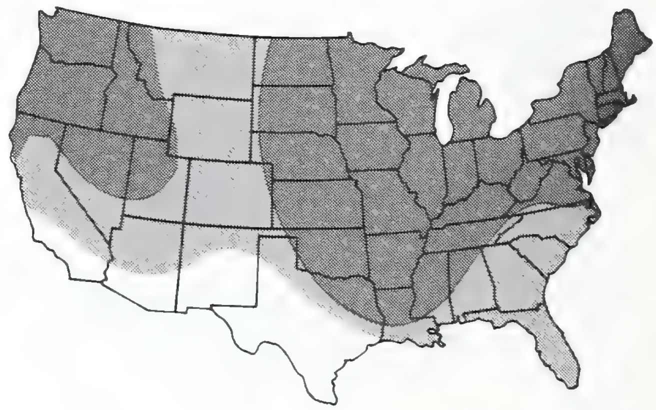


Figure 14.—Spring black stem, *Phoma medicaginis* Malb. & Roum. *(Ascochyta imperfecta).*

Figure 15.—Stemphylium leaf spot, *Pleospora herbarum* (Pers. ex. Fr.) Rab. *(Stemphylium botryosum* Wallr.).

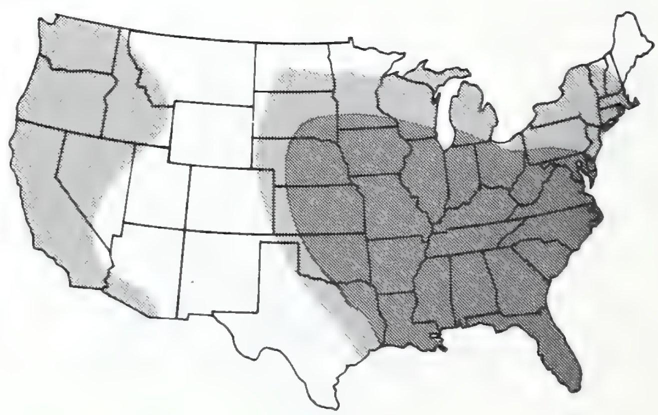


Figure 16.—Summer black stem, *Cercospora medicaginis* Ell. & Ev.

Figure 17.—Verticillium wilt, *Verticillium albo-atrum* Reinke & Berth.

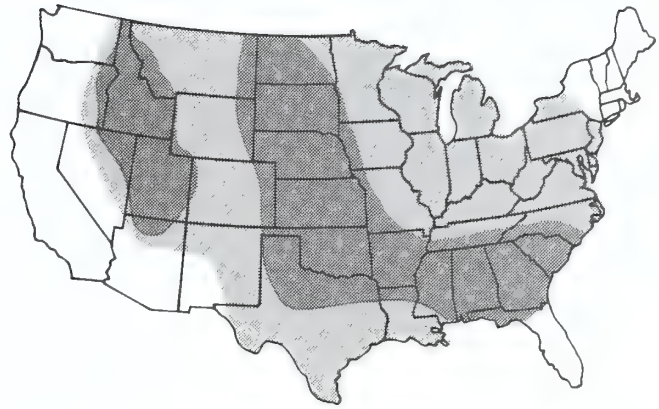


Figure 18.—Yellow leaf blotch, *Leptotrochila medicaginis* (Fckl.) Schuepp.

Figure 19.—Alfalfa mosaic virus.

## Distribution and Severity of Alfalfa Pests

### Insects and nematodes

- Not known to occur
- Occurs but is not an important problem
- Occasionally causes signficant losses on susceptible cultivars
- Frequently causes significant losses on susceptible cultivars

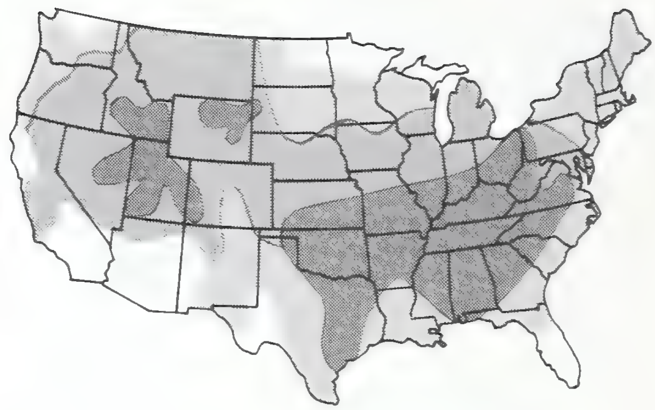


Figure 20.—Alfalfa weevil, *Hypera postica* (Gyllenhal).

Figure 21.—Egyptian alfalfa weevil, *Hypera brunneipennis* Boheman.

Figure 22.—Meadow spittlebug, *Philaenus spumarius* (Linnaeus).

Figure 23.—Pea aphid, *Acyrthosiphon pisum* (Harris).

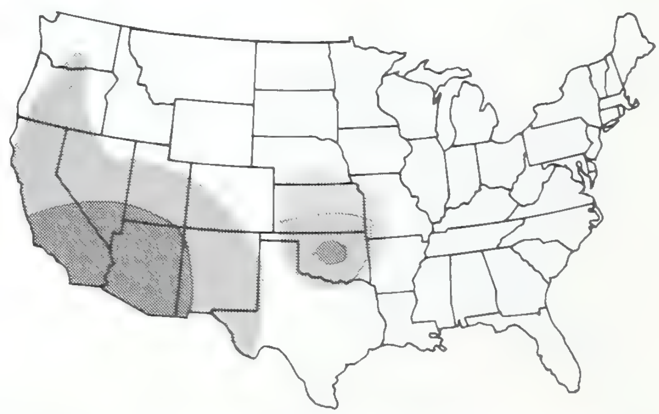


Figure 24.—Blue alfalfa aphid, *Acyrthosiphon kondoi* Shinji.

Figure 25.—Spotted alfalfa aphid, *Therioaphis maculata* (Buckton).

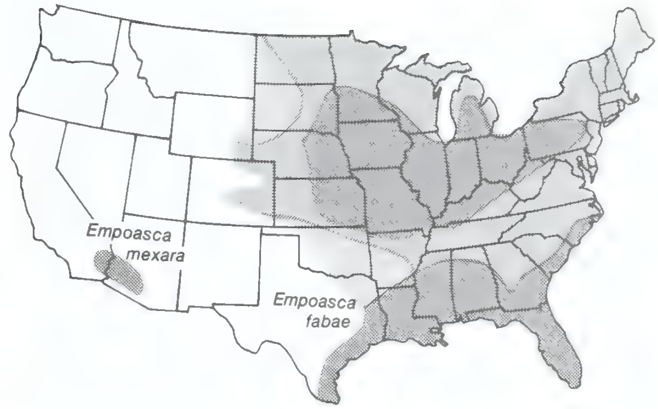



Figure 26.—Potato leafhopper, *Empoasca fabae* (Harris) and *E. mexara* Ross and Moore.

Figure 27.—Stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev.

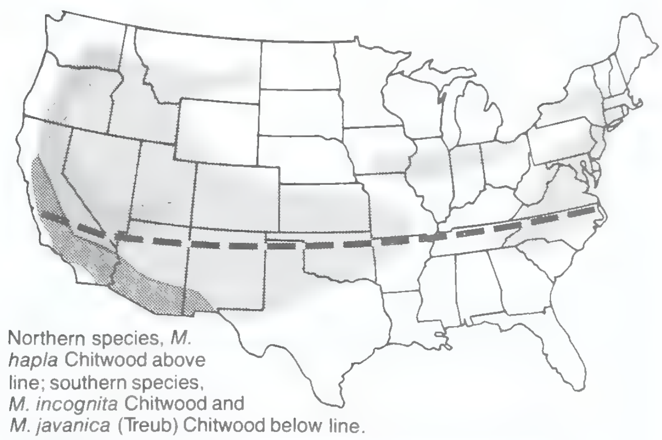



Figure 28.—Root-knot nematode, *Meloidogyne* sp.

## Established Cultivar Evaluation Procedures

Few examples of continuous long-term pest resistance tests are available for alfalfa. In fact, the oldest program in the United States is the 31-year-old bacterial wilt test conducted at the University of Minnesota. Many evaluation procedures for other types of pest resistance are more recent or have been used only sporadically. For these reasons, referring to these procedures as standardized evaluation tests would be presumptuous of the authors. Nevertheless, many of the tests can be used for cultivar evaluations.

Established evaluation procedures were available for 20 of the 36 major alfalfa pests listed in this publication. Scientists that had experience with these tests were asked to prepare brief descriptions of their methods. These descriptions should provide useful guidelines for future evaluations. Approximate levels of pest resistance expected for the check cultivars recommended for use with the evaluation procedures are given in the appendix, p. 37.

### Bacterial Wilt Resistance

F. I. Frosheiser and D. K. Barnes
University of Minnesota, St. Paul

The most reliable method of evaluating alfalfa cultivars for bacterial wilt (*Corynebacterium insidiosum* (McCull.) H. L. Jens.) resistance has been the bare-root soak method of inoculating the plants and then growing these plants for 3 to 4 months in the field. This method was developed by Cormack and others *(12)* and modified by Elling and Frosheiser *(20)*.

Bacterial wilt-infected roots, free of other diseases, are selected from previous field tests as inoculum. After the crown is removed, the roots are washed, ground, packed in plastic bags, and stored at −18°C *(50)*. Bacteria will remain virulent several years in continuously frozen material. About one-half hour before inoculation, the infected root material (50 g/L) is soaked in tap water to make a bacterial suspension. The ground-root material is left in the water throughout the inoculation period.

Seedling plants can be grown on any substrate before being inoculated. In Minnesota they are grown in sand for at least 8 weeks. Plants are inoculated with *Rhizobium meliloti*, fertilized and watered to promote maximum growth. Using sand simplifies pulling the plants and washing the roots. After the plants are pulled, they are individually separated and small ones discarded. From 50 to 70 plants per replication are tied in a bundle. Three replications are grown for each entry.

Roots must not be allowed to dry out before inoculation. Plants are inoculated by immersing the roots in the bacterial suspension for 20 to 30 min. Upon removal, the tops of the plants are cut off about 5 cm above the crown and the roots trimmed to about 10 to 12 cm. Several bundles are wrapped together in paper towels to keep the roots moist until transplanted. The inoculated plants wrapped in this manner and placed in 1 to 2 cm of water can be stored for several days at 2° to 4°C before transplanted.

Inoculated plants are transplanted to the field in early June with a modified tobacco transplanter and are spaced about 15 cm apart within the row with 1 m between rows. The field is cultivated and irrigated to maintain vigorous plant growth throughout the summer. Harmful insects may need to be controlled. During August, dead plants in the very susceptible cultivars are removed and recorded.

About 3 months after transplanting (Sept. 10–15), the plants are undercut about 15 cm deep, pulled, and the taproots sectioned and examined for internal discoloration. Ratings are based on a 0 to 5 scale as follows: 0 = sectioned surface is clean and white; 1 = very small spots of yellow-brown discoloration are visible in the stele; 2 = discoloration is more evident with up to one-third of stele affected; 3 = nearly the entire stele is discolored but the cortex is relatively white; 4 = discoloration extends throughout the stele but plant is still alive; and 5 = root is severely rotted with plant dead or dying. Classes 0 and 1 are considered resistant.

Levels of bacterial wilt resistance among cultivars within a test can be expressed as percentages of resistant plants or as ASI. The ASI score is more precise than percentage of resistant plants and is more useful in comparing relatively small differences in degree of resistance in a single test *(2)*. The percentage of resistant plants, however, is most useful in comparing cultivars evaluated in different test years. Data from each test are expressed as a percentage of a standard resistant check cultivar (Vernal). These data then are adjusted according to a longtime average (table 1). Nearly all cultivars grown in the United States have been evaluated at the University of Minnesota with these procedures.

**Table 1.—Percentage of bacterial wilt resistant plants of alfalfa cultivars compared with Vernal**

| Cultivar | Tests | Average of Vernal per test | Resistant plants per cultivar[1] |
|---|---|---|---|
| | *Number* | *Percent* | *Percent* |
| Agate | 2 | 154.0 | 65 |
| Iroquois | 2 | 145.3 | 61 |
| Narragansett | 15 | 1.5 | 1 |
| Ramsey | 4 | 89.6 | 38 |
| Ranger | 16 | 35.0 | 15 |
| Vernal | 16 | 100.0 | 42 |

[1]Calculated by multiplying percentage of Vernal by 42.0 (average percentage of resistant plants in Vernal from 16 test years).

**Anthracnose Resistance**

S. A. Ostazeski and J. H. Elgin, Jr.
Beltsville Agricultural Research Center, Beltsville, Md.

Alfalfa seedlings and mature plants react similarly when infected by the anthracnose fungus (*Collectotrichum trifolii* Bain). This simplifies the evaluations of resistance. Seedling evaluation procedures were first described by Ostazeski and others *(68)*. These procedures have since been refined and used to develop resistant populations *(13)* and cultivars *(14)*.

Until recently, only one race of the anthracnose fungus was known to occur. Since 1978, however, race 2 has been isolated from scattered fields of previously anthracnose-resistant alfalfa in North Carolina, Maryland, and Virginia *(70,71,89)*. The cultivars 'Arc' and 'Saranac' serve to differentiate the two races. Race 1 is virulent on 'Saranac' only, whereas race 2 is equally virulent on both cultivars. 'Saranac AR' is resistant to both races.

Both races are cultured identically. The inoculum source is obtained from isolates collected periodically from infected plants in the field. These can be increased on such culture media as oatmeal agar, potato dextrose agar, V-8 juice agar, or lima bean agar. Oatmeal agar is preferable. Optimum temperature for growth and sporulation in culture for most isolates is 25°C. Alternating on-off cycles of fluorescent light during incubation can enhance sporulation. Maximum sporulation is obtained from 7- to 10-day-old plates which were seeded with fresh spore suspensions. Poor inoculations often result when plate cultures older than 10 days are used for inoculum *(88)*. Spores are harvested by flooding plate cultures with about 10 ml of water supplemented with two drops of Tween 20/L. Spore concentration is adjusted to $1 \times 10^6$ spores/ml and is applied to run off with an atomizer, sprayer, or air brush. About 50 ml of inoculum are applied to each 30- by 60-cm flat of seedlings.

A dry inoculum method also has been developed *(9)*. Diseased plants from susceptible cultivars grown under controlled conditions are dried and ground. This inoculum is applied to seedlings as dust at a rate of 10 g/flat. Dry inoculum can be stored at least 10 months at $-20$°C without loss of virulence *(69)*. Dry inoculum should not be collected from field epiphytotics because it probably contains other pathogens, especially *Rhizoctonia*.

Seeds are sown in steamed soil in sterilized flats. Plant spacing is 3.8 cm between rows and 2.0 cm within rows. Before and after inoculation, seedlings are grown in a growth chamber at 23°C, with 16-h daylength and 17.2 klux light.

About 14 days after seeding, the seedlings are inoculated and moved to a dark walk-in incubation chamber similar to that described by Leath and Hill *(54)* and operated at approximately 23°C. Our chamber is humidified with a two-nozzle, compressed air-powered atomizer and operates 1 min of every hour.

As an alternate, a moist chamber can be fashioned directly over the plants with a wooden or pipe framework covered with polyethylene sheeting. More uniform humidity for infection is attained if plants are in a dark enclosure. Temperature should be 20 to 25°C. High humidity can be maintained with one or more humidifiers. Using such a setup on a bench in a darkened growth chamber required us to operate the humidifiers 2 consecutive minutes each half hour. Inoculated seedlings are held in the moist chamber for 48 h.

Plants are scored about 2 weeks after inoculation. Scoring is from 1 (resistant) to 5 (dead) and corresponds to lesion type *(68)*. Type 1 stems have no lesions or only small, water-soaked spots. Type 2 is a long, narrow lesion with few, if any, acervuli and no sporulation. Type 3 lesions are long, wide, not girdling, with acervuli usually present. Type 4 lesions are large, coalescing, and sporulating eventually girdling and killing the stems. In cultivar evaluations, plants with lesion types 1 and 2 can be classified as resistant. Percentages of resistant plants can be compared among cultivars by relating each cultivar to the standard resistant check. An ASI also can be used to compare cultivars.

Before initiating new programs on anthracnose resistance, other less conventional evaluation methods should be considered. Specifically, Graham and others *(26)* have described the agar plate method for selecting alfalfa resistant to *C. trifolii*. Cornmeal agar, in plates, is used to grow the fungus, for germinating seed, and for growing resistant seedlings until they can be transplanted to soil. The authors claim large populations can be screened in a minimum of time and space as effectively as with other methods.

Morrison *(58)* described a seedling box test for evaluating alfalfa for resistance to anthracnose in which plastic shoe boxes are used to grow, inoculate, and incubate seedlings. Resistance could be evaluated within 3 weeks of sowing seed.

**Common Leaf Spot Resistance**

**Laboratory and Greenhouse Methods**

K. T. Leath and R. R. Hill, Jr.
U.S. Regional Pasture Research Laboratory, University Park, Pa.

Cultures of the fungus *Pseudopeziza medicaginis* (Lib.) Sacc. are stored under refrigeration on oatmeal agar (36 g of Difco oatmeal agar preparation plus 7 g of agar/L of distilled water). These cultures are flooded with distilled water, and

the surface crust of the central fruiting structure, or apothecia, is fragmented to increase the inoculum. Approximately 1 ml of the suspension is distributed evenly over the surface of the oatmeal agar in a petri dish. About 50 plates are poured per liter of agar, and an increased ratio of about 1:10 can be expected. Usually two increases are required to obtain enough culture plates for an inoculation. Aseptic conditions are maintained throughout these increases. Cultures are incubated in the dark at 20°C for 21 days. At this time a sample plate is inverted over a microscope slide for 1 to 2 h. This slide is examined at 100× magnification to ensure that the cultures are sporulating.

Seedlings for inoculation are grown in metal flats filled with a 1:1 mixture of soil and peat. Seeds are planted on about 4-cm centers and are inoculated when the seedlings have 7 to 10 fully expanded leaves. The plants take approximately 30 days to reach that stage in the greenhouse.

Plants are moved to an inoculation-incubation chamber *(54)* that is maintained at 20°C in the dark with saturated relative humidity. Sporulating cultures are inverted and suspended about 20 cm above the tops of the plants. The dishes are spaced on about 15-cm centers and rearranged periodically to ensure uniform spore coverage. The spores are small enough for some lateral dispersion to occur. Cultures are allowed to deposit spores for about 8 h before the plant surfaces are sprayed with a fine mist of distilled water, which is terminated just before runoff. Cultures are left to deposit spores on the plants for 48 h. Plants are retained in the moist chamber an additional 24 h and are allowed to dry slowly before returning them to the greenhouse. Because the plants have been in the dark at a high humidity, they should not be exposed immediately to full sunlight. Plants should be moved into subdued light and then, during late afternoon, should be moved into the greenhouse.

At temperatures of 20° to 26°C, symptoms develop about 3 weeks after inoculation. Resistance is determined by the size of lesions, the presence or absence of the apothecium, and the leaf chlorosis and defoliation. We recommend a 1 to 5 scale for scoring disease severity. The classes are 1 = no spots; 2 = very small black spots without apothecia; 3 = small black spots with small apothecia; 4 = large black or brownish spots with large apothecia; and 5 = large black or brownish spots with large apothecia accompanied by chlorosis and defoliation.

Plants rated 1 and 2 are considered resistant. When leaves are heavily infected, spots may not develop normally. Thus, leaves that are densely covered with class 2 spots may not be truly resistant. Usually, heavily infected leaves of susceptible plants will turn yellow and drop off. The chlorosis that is typical of class 5 susceptibility on heavily infected susceptible leaves may be more striking in the field than in the greenhouse. A susceptible check is useful in estimating the frequency of escapes. A resistant check is used to compare results between tests.

### Field Methods

F. I. Frosheiser
University of Minnesota, St. Paul

If plants are spaced about 30 cm apart in the row and about 60 cm between rows individual plants can be examined with space to maneuver between rows. Wider spacing may result in less infection. Alternate rows of a susceptible cultivar may be useful in increasing disease potential but are usually unnecessary. Natural epiphytotics of common leaf spot usually occur in Minnesota and adjacent States during late summer or early fall. These epiphytotics are often relatively pure and do not contain other diseases, especially in first-year plantings. Plantings made in early May should be clipped once in mid-July and then allowed to mature until an epiphytotic develops.

Each plant should be examined individually and rated on a 1 to 5 scale. The same scale recommended for laboratory testing can be used in field evaluations. In 1971 Minnesota field tests, the cultivars 'DuPuits', 'Cayuga', and 'Atlantic' were scored for common leaf spot resistance along with 107 other cultivars. Percentages of resistant plants for the three cultivars were 'DuPuits', 76; 'Cayuga', 40; and 'Atlantic', 14, compared with 'DuPuits', 80; 'Cayuga', 32; and 'Atlantic', 20 percent as reported by Graham and others *(28)* in greenhouse tests. Both laboratory and field tests appear suitable to evaluate cultivars.

### Lepto Leaf Spot Resistance

K. T. Leath and R. R. Hill, Jr.
U.S. Regional Pasture Research Laboratory, University Park, Pa.

Cultures of the fungus *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell are stored in soil under refrigeration. Cultures for inoculum are started by depositing soil crumbs containing the fungus onto plates of vegetable juice agar *(57)*. All cultures are grown at 21° ± 1°C under cool-white fluorescent light, at about 500 lux, or preferably under long-wave ultraviolet light (UV) (BLB40 lamps, 30 cm above the plates). More ascospores are produced with the near-UV lamps *(53)*, but cool-white lamps will suffice. The resulting fungus cultures are used as starter plates for increasing inoculum.

These cultures are flooded with sterile distilled water, and the perithecia are manually scraped loose from the agar. Approximately 1 ml of the fungal suspension is distributed over the surface of the agar in each plate to be used as inoculum. About 50 plates are poured per liter of agar, and an

increase ratio of about 1:10 is usually obtained. Two increases may be necessary to get from soil storage to a large-scale production of inoculum. After about 72 h, ascospores are present in the cultures and can be seen readily on the lids of the petri dishes at 100 × magnification. Plates are usually 72 h old when they are used to inoculate plants.

Plants for inoculation are grown in metal utility carts filled with a 1:1 mixture of soil and peat. We plant on about 4-cm centers and inoculate when the plants have 7 to 10 fully expanded leaves, which takes about 30 days in the greenhouse. Because vigorously growing plants are necessary to get susceptible host-plant responses to infection *(55)*, we grow our plants under metal halide lamps that provide the 20,000 lux at plant height needed for true symptom expression.

Plants are placed in an inoculation-incubation chamber *(54)* that is maintained at 20°C in the dark with saturated relative humidity. Sporulating cultures are inverted and suspended about 20 cm above the tops of the plants. The plates are spaced on about 15-cm centers and must be rearranged frequently to ensure uniform inoculations. Lateral dispersal of falling spores is minimal. Cultures are allowed to drop spores until a spore density of 10/cm$^2$ on trap slides is reached. At this time, usually 1½ to 4 h, plates are removed, and the plants are sprayed with a fine mist of distilled water until leaf surfaces are wet. Plants are retained in the moist chamber for 30 to 48 h and are allowed to dry slowly before being returned under the lights in the greenhouse. When the plants are returned to the greenhouse, the light intensity must be maintained at the minimum 20,000 lux because susceptible symptom expression does not occur under low-light, slow-growth conditions.

Symptom development is rapid with this disease. Often chlorotic flecks are visible when the plants are removed from the moist chamber. Resistance is determined by the size and type of lesions at about 10 days after inoculation. We recommend a 1 to 5 scale for rating disease response. The classes are 1 = no spots; 2 = very small black spots; 3 = large black or small tan spots without chlorotic halos; 4 = small tan spots with halos and large tan spots without halos; and 5 = large tan spots with pronounced halos and coalesced spots forming blotched leaf areas. Plants rated 1 and 2 are considered to be resistant. Heavy inoculations should be avoided because spots do not develop normally, and leaflets tend to desiccate and abscise. A susceptible check cultivar is useful in estimating the frequency of escapes as well as the suitability of environmental conditions. A resistant check should be used to compare results between tests.

## Downy Mildew Resistance

### Laboratory Methods

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Physiological races of *Peronospora trifoliorum* d By. occur; therefore, a diverse collection of conidia (sporangia) is recommended for screening. Conidia from field plants usually are contaminated with bacteria and fungi and germinate poorly. Producing inoculum for tests on seedlings in the laboratory is preferable. To inhibit contaminants, add 50 $\mu$g nystatin and 10 $\mu$g tetracycline/ml of inoculum *(83)*. The following schedule permits using conidia from infected seedlings in a test as inoculum for the next test. We prefer, however, to include separate flats seeded thickly with susceptible alfalfa from each test to produce inoculum for the next test. Conidium germination is greatest when plants are harvested and placed immediately in water 12 to 16 h after they are covered to induce sporulation. Conidium viability declines rapidly if harvest is delayed or if conidia are exposed to dry air for more than a few seconds *(23)*. A low percentage of conidia, however, will survive for a few weeks on diseased seedlings stored at −20°C. Conidia will remain viable for many years in liquid nitrogen *(7)*.

To prepare inoculum, excise seedlings or leaves with sporulation and place immediately into a jar containing chlorine-free water *(22)*. Shake the jar vigorously to dislodge conidia and pour the spore suspension through a tea strainer to remove plant debris. To inoculate, spray the spore suspension onto seedlings to point of runoff. Uniform infection requires at least 25,000 viable conidia/ml of inoculum. Prevent plants from drying for 12 h by placing them under an airtight cover. The same covering procedure is used to induce sporulation, which requires darkness and nearly 100 percent relative humidity *(23)*.

Plants are maintained in growth chambers at 20°C and 5,000 to 10,000 lux of continuous fluorescent lighting throughout, except for designated dark periods. We use the following schedule routinely as it requires little attention on weekends.

Day 1
(Friday)
Plant seeds in 1.3-cm-deep rows in flats of fine sand. We space two to three seeds/cm within rows 2.5 cm apart.

Days 3 to 5
Sprinkle water on flats twice daily to settle the sand around the seedlings to insure an even emergence.

Day 5
(Tuesday)
Seedlings are emerged, and cotyledons are expanded. Spray on inoculum, cover plants, and turn off lights for at least 12 h (overnight).

Day 6
(Wednesday, a.m.)
Uncover plants, turn on lights, and rogue any plants emerged since inoculation.
Days 7 to 10
Continue roguing newly emerged plants.
Day 11
(Monday, p.m.)
Cover plants again and turn off lights for 12 to 16 h (overnight) to induce sporulation. Do not water plants just before covering because the fungus will not sporulate in free water.
Day 12
(Tuesday)
Evaluate plants.

Evaluation of cultivars is based on the percentage of mildew-free plants compared with standard resistant check cultivar ('Saranac') included in each flat. We have noted considerable variability in the virulence of some of our *P. trifoliorum* isolates, however, at least 15 percent of the 'Saranac' plants are resistant to any isolate we have tested. A minimum of four 50-plant replications is recommended for each plant line tested.

**Field Methods**

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Spaced plants in the field also can be used to evaluate levels of downy mildew resistance in alfalfa cultivars *(73)*. Field epiphytotics are usually observed on succulent vegetative growth during early spring or late fall.

In the field, individual plants can be classified according to degree of resistance. A 4-class scale has been used at Minnesota and Utah (1 = no symptoms; 2 = small, usually nonsporulating lesions on one or two leaves; 3 = sporulating lesions on 10 to 25 pct of the leaves; and 4 = general infection over the entire plant). Plants classified as 1 or 2 are considered resistant. Cultivars can be compared statistically with susceptible and resistant check cultivars by either ASI or percentage of resistant plants.

### Fusarium Wilt Resistance

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The method developed to evaluate resistance to fusarium wilt is similar to that used for bacterial wilt, except that laboratory cultures of the pathogen are used as inoculum instead of infected host tissue *(25)*. *Fusarium oxysporum* Schlecht f. sp. *medicaginis* (Weimor) Snyd. & Hans. can be isolated readily from diseased roots on acidified potato dextrose agar. Stock cultures are maintained in sterile soil in culture tubes. The fungus remains viable and retains its pathogenicity for long periods (several years) when maintained in this manner even though the soil becomes dry.

Inoculum is increased by growing the fungus in nutrient broth prepared by dissolving 2 g $NaNO_3$, 1 g $KH_2PO_4$, 0.5 g $MgSO4{\cdot}7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4{\cdot}7H_20$, 0.5 g yeast extract, and 15 g sucrose in 1 L of distilled water and autoclaving for 20 min at 121°C. Potato broth may be substituted for nutrient broth. Wash and slice 300 g potatoes and boil in water for 15 to 20 min. Strain through four layers of cheesecloth and add water to make 1 L. Autoclave for 20 min at 121°. Add a small amount of infested soil from stock cultures to the broth and incubate on a shaker for 4 days at room temperature (21°). At higher temperatures the incubation period may be shortened. Abundant microconidia and minimum mycelial fragments are produced. The spore suspension is diluted with water to a concentration of $1.5 \times 10^6$ spores/ml. If facilities for estimating the spore concentration are lacking, the concentrated inoculum may be diluted 1:20 to obtain the proper concentration.

Alfalfa seedlings are grown in sand in greenhouse benches. They are inoculated with *Rhizobium meliloti* and fertilized and watered to promote optimum growth. The plants are lifted when 10 to 12 weeks old, the roots are washed in tap water, and about 50 plants for each plot are tied in a bundle. The roots are kept in tap water until all plants for all plots in a replicate are prepared. Then the roots are immersed in the inoculum for 20 to 30 min. After inoculation, the tops are trimmed to about 4 cm from the crown and the roots are trimmed to about 12 cm. The bundles are arranged into the planting order, and five are wrapped together in paper toweling and set upright in a container with 1 to 2 cm of water to keep the roots wet until transplanting. If not planted immediately, inoculated plants may be stored at 3° to 4°C for several days. The plants are transplanted about mid-June, using a modified tobacco transplanter, into the field in single-row plots, spaced about 20 cm apart in rows spaced about 1 m apart. The number of established plants is recorded 12 to 14 days after transplanting. Many of the susceptible plants are dead within 5 to 6 weeks.

The surviving plants are evaluated about 3 months after planting. They are undercut and lifted, and each taproot is sectioned and rated for disease severity. The ratings are based on a 0 to 5 scale: 0 = no discoloration in the root; 1 = small dark strands in the stele; 2 = small dark-brown arcs or rings in cross section of the stele; 3 = larger dark-brown areas, arcs or rings, or partial dark-brown ring in outer stele; 4 = entire outer stele dark brown, plant alive; 5 = plant dead (calculated as loss in stand count from 2 weeks after transplanting). Plants rated 0 and 1 are con-

sidered resistant. The level of fusarium wilt resistance among cultivars within a test can be expressed as the percentage of resistant plants or as ASI. Disease ratings can be compared among cultivars by relating each cultivar to the standard resistant check cultivar. The same procedure can be used with acceptable accuracy in the greenhouse by transplanting inoculated plants into pots or benches.

### Phytophthora Root Rot Resistance

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#### Field Methods

Select a level area with relatively heavy soil. If the soil is not naturally infested with *Phytophthora megasperma* Drechs. f. sp. *medicaginis*, infested soil may be spread over the area and incorporated into the upper 15 cm. Infested soil can be from naturally infested fields or from greenhouse experiments.

Seed is planted in early May at about 75 viable seeds per 1.6 m of row in rows about 30 cm apart. A minimum of three replications per entry is suggested. A stand count is taken approximately 2 weeks after planting while the seedlings are in the unifoliolate leaf stage. After the seedlings are about 4 weeks old, rainfall must be supplemented with irrigation to keep the surface soil continuously at or near saturation for 2 to 3 weeks. After the first 2- to 3-week wet period, the soil is allowed to dry. This allows time to take notes, clip, and cultivate. If the disease is severe, the plants may be evaluated at this time; however, one, and preferably two, additional 3-week wet periods reduce the frequency of escapes. These additional periods provide a more accurate evaluation of the degree of root rot on each plant because the roots are larger. Plants are dug and scored during the first 2 weeks of September.

When plants are dug, as much of the root system as possible should be retained. At least 30 cm of taproot are needed. All the plants from a row are dug and tied in a bundle. The roots are soaked in a tub of water and then are washed with water under pressure. Plants are individually classified for degree of root rot. We prefer to do all classification under a uniform light source in the greenhouse rather than in the field.

A 1 to 6 classification scale described by Frosheiser and Barnes *(24)* is used: 1 = roots clean with no lesions and many small rootlets present on the taproot; 2 = only very small superficial lesions (2 mm) present on taproot, taproots usually lack numerous branch roots, and most lesions occur at site where branch root had started growth; 3 = one or more large lesions on taproot, but none girdling the taproot, the tips of one or more larger branch roots rotted off; 4 = extensive root lesions with the taproots usually rotted off 10 cm or more below the crown; 5 = taproot almost completely destroyed, but plant alive; and 6 = plants dead (calculated as loss in stand count from 2 weeks after seeding to mid-Sept.). Plants rated 1 and 2 are considered resistant. All tests should contain a resistant check and a susceptible check.

Level of resistance among cultivars within a test can be expressed as percentage of resistant plants or as ASI. Percentage of resistant plants can be used to compare cultivars in different tests. This procedure was described previously in the bacterial wilt evaluation procedures and consists of expressing the data from each test as the percentage of the resistant check cultivar, 'Agate'. The data are adjusted to 'Agate's longtime average of 43 percent resistant plants. Most cultivars grown in the United States have been tested at the University of Minnesota.

#### Greenhouse Methods

Greenhouse tests are conducted in 20-cm-deep, watertight containers filled with fine, steamed sand *(24)*. The sand is infested initially by using laboratory cultures. The fungus is grown on V-8 juice agar *(57)* (200 ml V-8 juice, 20 g agar, 1 g $CaCO_3$ in 800 ml water) in 9-cm petri dishes. Two-week-old cultures are blended in water and then added to the sand in one petri dish to 500 $cm^2$ surface area. The inoculum is mixed with the sand.

Seed is planted directly into the sand, and the seedlings are watered sparingly until they are well established (about 4 weeks). The drain holes in the tanks are plugged, and enough water is added daily to raise the water level to the sand surface. Sand temperatures of 20° to 24°C are optimum for Phytophthora root rot. After about 4 weeks of continual flooding, the plants can be evaluated for root rot as described in field evaluations. Resistant and susceptible check cultivars should be included in each test for comparisons.

Except in unusual cases, no additional inoculum needs to be added to the sand for repeated testing. Field and greenhouse evaluations were correlated (r = 0.99 and 0.95) in two tests *(24)*. We think field tests give a more accurate evaluation of cultivars, however, because the roots are larger and easier to score and more plants can be observed. Nevertheless, greenhouse procedures are useful for screening and are being used successfully by a number of plant breeders *(30)*.

## Verticillium Wilt Resistance

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Laboratory cultures of the alfalfa strain of *Verticillium albo-atrum* Reinke & Berth. are used in root-soak techniques for the greenhouse, growth chamber, or field. This organism is isolated from infected leaves or stems that show distinct symptoms. The best stems are still green and show a yellow streak or partially chlorotic leaves. The tissue is surface sterilized in 70 percent alcohol for 5 to 15 s (depending on the thickness of plant material), rinsed in sterile distilled water, blotted on sterile filter paper, placed on water agar in petri dishes, and incubated at 20° to 25°C for 2 to 5 days. Transfer is then made to prune agar *(85)* and the cultures are incubated for 10 days at 20° to 25°. Conidia are floated off the dish in sterile distilled water to produce 7 to 10 ml of inoculum. If a counting devise is available, the concentration is adjusted to approximately $8 \times 10^6$ conidia per ml. (A hemacytometer works very well.)

Stock cultures may be maintained on silica gel *(74)*. Difco Czapek Dox broth can be used to produce large quantities of *Verticillium* inoculum more efficiently if a shaker is available. A small amount of fungus is transferred from prune agar to 50 ml of sterile Czapek broth in a 125-ml flask. It is placed on a shaker at 120 to 130 r/min at 20° to 25°C, for 7 to 10 days. The contents are then filtered through two layers of cheesecloth and centrifuged at 3,800 × G for 20 min. The supernatant is discarded and the pellet resuspended in sterile distilled water and diluted to a conidial concentration of approximately $8 \times 10^6$/ml. Inoculum can be stored at 5° for a week or more. Each flask yields 200 to 400 ml of dilute inoculum. The centrifuge procedure, though not absolutely necessary, produces a cleaner inoculum with a higher percentage of conidia than that strained through cheesecloth.

Roots of 4- to 12-week-old plants are washed; cut to a convenient length or injured, if already short enough; and soaked in the inoculum for about 5 min (time is not critical). Top growth of older (>8 weeks) plants is trimmed. Twenty-five plants per entry per replication are transplanted into flats or pots in the greenhouse or growth chamber at 20° to 25°C and plants can be rated for disease severity in 3 to 5 weeks. Fresh inoculum usually is used for each replication. Inoculated plants transplanted into the field require the full growing season for best results. Rating is on a scale of 1 to 5 with 1 = no symptoms; 2 = 1 or 2 leaflets showing slight mottling but no distinct symptoms; 3 = distinct symptoms; 4 = severe symptoms; 5 = dead plants. Stunting is not considered in the rating. Plants rated 1 and 2 are classified as resistant, and results can be expressed as percentage resistant plants or all data can be used to compute an ASI. Susceptible checks, such as 'Vernal' or 'Saranac', normally show less than 5 percent escapes. 'Vertus' contains approximately 45 percent resistant plants.

## Rust Resistance

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Laboratory methods are most suitable for evaluating rust (*Uromyces striatus* Schroet.) resistance in alfalfa although several researchers have successfully screened and evaluated germplasm in the field *(11,15)*. One laboratory method, the detached leaflet-petri dish technique, was described earlier *(39)* and is useful for evaluating small numbers of plants. For larger numbers of plants, however, the following techniques are recommended.

To minimize the possibility of culturing a single physiological race of rust, urediospores should be collected from diseased plants in late summer and fall from a number of field locations *(39)*. Initial collection may be done by applying light suction to a cyclone spore collector similar to that used with *Puccinia* in cereal rust *(8)*. Flexible tubing is attached to the collector providing an opening for oral suction, and the bottom half of a 33- × 8-mm gelatin capsule is used as a receptacle to retain the spores. The capsule is capped with the top half for storage. Urediospores can be collected and stored dry at -8°C for 12 months with little loss of viability. Alternate methods of collecting urediospores are by brushing, tapping, or shaking diseased leaflets over funnels or pans. Spores also may be stored in envelopes or other suitable containers.

The inoculum usually does not have to be increased unless small numbers of plants (<50) are to be tested. This is most easily done on vigorously growing susceptible plants. Plants may be inoculated by dusting, brushing, or rubbing urediospores onto leaflets, and small numbers of plants can be inoculated with the cyclone-spore collector by reversing the airflow. Diluting the spores with dry talcum powder enhances dispersal of small quantities of inoculum *(56)*. For inoculating larger numbers of plants, a larger duster can be fabricated from a small jar, an atomizer bulb, a rubber stopper, and glass and rubber tubing.

A substantial supply of urediospores may be obtained by inoculating flats of a susceptible cultivar and collecting urediospores from the plants by shaking them over an enamel tray. Slanting the tray slightly and tapping the bottom lightly result in the gravitational movement of any soil, leaves, and nonspore particles to a corner of the tray where they can be removed. The urediospores are then tapped to the corner of the tray and are scooped into a container for storage.

Approximately 4,000 plants can be inoculated with 0.45 g of urediospores. Seedlings for evaluation are grown in the

growth chamber or greenhouse in 21- × 62-cm wooden flats with 3.8 cm on center spacing, approximately 128 seedlings per flat. When 4 to 5 weeks old, the seedlings are placed in a 2.4- × 2.4- × 1.8-m dark incubation chamber similar to the one described by Leath and Hill *(54)*, except that moisture is provided by an air pressure mist atomizer operated once daily for 5 min. The chamber holds 18 flats. Chamber temperature is maintained at 22°C. Plants are sprayed with tap-water mist and then inoculated with a dry dust suspension of 0.45 g of urediospores in 8.9 g of talcum powder. A pressure pump at 12 to 15 psi is used on a small jar duster for inoculation. Counts with a hemacytometer verify that approximately 100 million spores settle in the area of the chamber in the dust suspension. The seedlings are removed from the incubation chamber after 48 h.

The most desirable time for rating the diseased plants is 16 to 20 days after inoculation. The most severely infected leaflet of each plant is scored for development of pustule size. Scoring is on a scale from 1 to 5. Class 1 = no symptom development; 2 = leaf flecks, possibly with a few small closed pustules; 3 = a few flecks and closed pustules plus several small open pustules; 4 = many small open pustules; and 5 = many medium to large open pustules. Score classes 1 and 2 are considered resistant since these plant types inhibit pustule development. In score classes 3 and 4, some pustule development is inhibited, whereas the plant types in score class 5 promote complete pustule development and abundant spore production. Percentage resistant plants or ASI of cultivars can be used for comparisons with check cultivars.

### Yellow Leaf Blotch Resistance

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*Methods used at South Dakota State University, Brookings*

Greenhouse techniques are used to evaluate for resistance to yellow leaf blotch caused by *Leptotrochila medicaginis* (Fckl.) Schuepp (77). Seeds of entries to be evaluated are planted in a nonsteamed loam soil-sand mixture (2:1) in 7.6- x 7.6-cm greenhouse plant bands, 30 to a wooden flat. Seedlings subsequently are thinned to one per band, and plants are allowed to grow several months at 20° to 25° C with several prunings to promote branching. A minimum of 100 plants per cultivar is recommended, divided among at least four replications.

Inoculum consists of naturally diseased leaves (40 to 50) collected from the field and placed between 10- × 15-cm sheets of plasticized fiber glass window screening. The leaves between the screens are held outdoors from early summer to late fall until apothecia develop and mature on them *(76)*. For inoculations, the diseased leaves, still between the screens, are wetted and the screens are arranged close together over plants to be inoculated. This arrangement may involve as few as 12 and as many as 35 flats at one time within a frame tightly covered with clear, 3-mil polyethylene sheeting. Protection from direct sunlight is provided, and greenhouse temperatures are held at 20° to 25°C. The diseased leaves between the screens are held over the plants for 48 h, and the plastic sheeting is removed 24 h later.

Plants are scored for disease symptoms between the second and third week after uncovering them. Because of possible nonuniform inoculations, they are scored for symptom type, not for abundance of infected leaves. Leaves are rated on a scale from 1 to 5: 1 = no evident infection; 2 = small, dark circular spots with no chlorosis; 3 = small, dark circular spots with chlorosis; 4 = one or more chlorotic leaf sectors extending from the leaflet edge to the midrib, with incipient pycnidia; and 5 = most to entire leaflet chlorotic with robust pycnidia and leaf curling. Resistance can be expressed as percentage resistant plants or as ASI. Lesion-type scores 1, 2, and 3 together are considered resistant; 4 and 5 are susceptible.

### Alfalfa Seed Chalcid Resistance

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Evaluations of seed chalcid (*Bruchophagus roddi* Gussakovsky) resistance can be conducted either in the field or in the greenhouse. Field evaluation of cultivars begins by establishing plants in rows spaced 1 m apart, with 50 to 60 cm between plants within rows. Six replications are usually used. Fields are managed so that plants are setting seed during August when alfalfa seed chalcids reach maximum populations. Samples of 25 racemes, with fully developed pods, are taken from each plant and are placed in a pint-sized ice cream carton. Adult chalcids are allowed to emerge. Statistical comparisons of cultivars are made on the basis of the mean number of adults per 25 racemes per plant *(65)*.

Greenhouse evaluations are made by caging 10 adult chalcids on a raceme. From 6 to 8 racemes are caged per plant. The adult chalcids are reared from infested seed screenings held in storage at 10°C. After 2 weeks, the caged racemes are removed and placed in rearing cartons. The number of emerged adult chalcids are counted after another 2 weeks *(60)*. As with field procedures, cultivars are compared by mean number of emerged adults per plant; however, cultivars also are rated according to percent resistant plants. Resistant plants are those that average no more than one adult per raceme.

## Alfalfa Weevil-Feeding Resistance

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Methods are available for laboratory selection of germplasm with alfalfa weevil (*Hypera postica* (Gyllenhal)) larval antibiosis and nonpreference to adult feeding and oviposition. Laboratory methods for evaluating larval antibiosis have been used successfully in selecting plants with a high level of resistance in several *Medicago* species *(4,78)*. Present levels of antibiosis and nonpreference in *M. sativa*, however, are too low to recommend use of laboratory methods for cultivar evaluation. These methods do not accurately identify tolerance to larval feeding, which is the principal mechanism of resistance in *M. sativa* evaluated to date *(3)*. In field tests, weevil-resistance selections have been separated consistently on the basis of larval-feeding damage. Thus, field testing in areas with natural weevil infestations is the only practical method for evaluating cultivars for weevil resistance. Tolerance may be related to fall and spring growth characteristics plus the plant response to weevil injury.

Test entries should be seeded in broadcast or drill-row plots at least 4.5 $m^2$. Spaced plants have been unsatisfactory for alfalfa weevil evaluations. At least four replications of each entry, including resistant and susceptible check cultivars, should be included in each trial. Larval feeding damage is evaluated by estimating the percentage of leaves eaten (percent defoliation) on the basis of total leaves available. Percentage defoliation is estimated for the entire plot. Data for each entry should then be converted and presented as percent defoliated and compared with the resistant check, using a base of 100 for the check. An example of this procedure is shown in table 2. More than one evaluation date is necessary to determine the performance of most entries accurately.

**Table 2.—Alfalfa cultivars classified for tolerance to alfalfa weevil larval feeding by percent defoliation in relation to the check cultivar, Team, Raleigh, N.C., specified dates, 1970**

| Cultivar | Defoliation (pct of Team)[1] April 22 | May 1 | May 6 | Average |
|---|---|---|---|---|
| | *Percent* | *Percent* | *Percent* | *Percent* |
| Arc | 106 | 73 | 90 | 90 |
| Saranac | 186 | 172 | 146 | 168 |
| Atlantic | 224 | 195 | 151 | 190 |
| Cherokee | 236 | 213 | 151 | 197 |
| Percent defoliation of Team | 28.3 | 36.7 | 51.7 | 38.9 |

[1]Team = 100.

Conditions that influence plant growth and insect infestation levels cannot be controlled in the field; therefore, at least 2 years of data are obtained on each set of plots. The variation because of differences in population levels of alfalfa weevils (both seasonal and between locations) is reduced when tests are located in areas of known high weevil infestation. This reduction, however, often necessitates testing many lines or cultivars outside their area of development and adaptation, which can influence tolerance ratings. Recommending one or two specific test locations for alfalfa weevil testing, therefore, is difficult. Entomologists and plant breeders involved in developing resistant cultivars will need to determine appropriate test locations based on weevil pressures and cultivar adaptation.

Techniques similar to those used for alfalfa weevil feeding resistance can be used for evaluating Egyptian alfalfa weevil feeding resistance *(84)*.

## Pea Aphid Resistance

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Several methods and resistance criteria have been effective for evaluating resistance of alfalfa cultivars to the pea aphid, *Acyrthosiphon pisum* (Harris) *(10, 37, 67,* and *81)*. Plant resistance to pea aphid has been shown to be similar at various stages of growth in both laboratory and field. We use a seedling test under controlled conditions.

Numbers of aphids needed to evaluate resistance can be increased in the greenhouse on succulent susceptible alfalfa or on broadbeans, *Vicia faba* L. (*Faba vulgaris* Moench). Broadbeans, however, should be used with caution because aphids may become conditioned to them, which could in time influence their reaction on alfalfa.

Evaluation tests are conducted in either a growth chamber or greenhouse. Greenhouse tests are most practical, but aphids tend to congregate at the sides or ends of flats as temperatures and light intensities change. Tests conducted in growth chambers at 20°C, 55 to 65 percent relative humidity with a 16-h photoperiod at 13,455 lux of light, are more precise than greenhouse tests.

Seedlings are established in rows in greenhouse flats. A minimum of four 50-plant replications is recommended. Scarified seeds are planted on steam-sterilized soil and covered with 1.3 cm of sand. Flats are placed in aluminum trays for subirrigation. Seedlings are counted in the cotyledon stage and immediately infested by shaking large numbers of aphids over them. Additional aphids are added as needed to maintain a high population. Plants and insects are enclosed in 25-cm-high glass cages with plastic screen tops. When most plants of the susceptible check cultivar are

dead, the test is terminated and surviving seedlings in each row are counted. Resistance is expressed as percentage of seedling survival and is compared with that of a resistant check cultivar. In some Southwestern States, large field cages have been used successfully for aphid evaluations.

**Blue Alfalfa Aphid Resistance**

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Techniques for evaluating resistance to the blue alfalfa aphid, *Acyrthosiphon kondoi* Shinji, in the greenhouse are similar to greenhouse tests for the pea aphid and spotted alfalfa aphid *(63, 64)*. Because the blue aphid is difficult to rear in sufficient quantities in the Southwest, even during the cooler months (Dec.-April), the best results often are obtained with a field-cage technique. Rows of the susceptible entry PA-1 (resistant to pea aphid and spotted alfalfa aphid) are planted in the fall. The length and number of rows are determined by the size of the cage. In the spring, the field plot is caged; then the rows are infested manually to start the culture. When the population begins to build up, the test entries are planted between the PA-1 rows. After the test entries germinate, the PA-1 rows are cut back to force the aphid populations to feed on the young seedlings. The number of test entries that germinated are counted before cutting the PA-1 rows; then, the number of plants that survived after the susceptible check planted with the test entries is destroyed are counted. Resistance is expressed as percentage of seedling survival.

**Spotted Alfalfa Aphid Resistance**

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Seedling tests used for evaluating spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) resistance are similar to those used for pea aphid evaluations. Although resistance tends to increase in mature plants, resistance ratings from seedling cultivar evaluations are much like those of mature plants *(40, 41)*. Tests described here are similar to procedures suggested by Hackerott and others *(36)* and Klement and Randolph *(52)*.

All entries are planted in galvanized flats. Each flat contains 13 rows, 50 seeds to a row; 8 rows are test entries, and 5 rows are susceptible checks. The rows are arranged so that each test row is next to a susceptible check row. Each test includes four replications of each entry.

Seedlings are counted in the unifoliolate stage (approximately 7 days old) and infested manually with 4 $cm^3$ of aphids per flat. Tests are conducted in the greenhouse at temperatures between 20° to 30°C. When all plants in the susceptible check rows are dead, surviving seedlings are counted in each test entry. Resistance is expressed as percentage of seedling survival. Test entries are compared with a resistant check included in each test.

Nielson and Don *(61)* and Nielson and others *(62)* reported the existence of biotypes of spotted alfalfa aphid in the Western United States. Cultivars were evaluated for resistance to four biotypes by Nielson and others *(66)* using these techniques. If evaluations are conducted with aphids of unknown biotypes, a description of when and where the aphids were collected is useful.

**Potato Leafhopper Yellowing Resistance**

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Field tests to select for resistance and to evaluate materials for resistance to potato leafhopper (*Empoasca fabae* (Harris)) yellowing have usually been more successful than greenhouse seedling tests *(45, 47, 48, 49, 51, 59, 86, 87)*. Natural field infestations and manual infestations of field cages have been successfully used to evaluate the resistance of breeding materials and cultivars. The mechanisms of resistance are not well defined.

Spring seedings are made from mid-April to early May. Greenhouse-grown seedlings 6 to 8 weeks of age are transplanted to the field in late April or early May. Single row plots are used with a minimum of four replications. Although transplanted plots consist of 25 plants spaced 30 cm apart, spacings of 30 to 90 cm between rows have been successful. Seeded plots 3 to 5 m in length have also been used successfully, as have spring-seeded cultivar tests using multiple-row plots. Plantings are kept weed free and insecticides are not used.

The initial growth from spring transplanting or seeding can usually be scored for yellowing (or reddening, depending on plant genotype) by mid-July *(48)*. After the first cutting in the year of establishment and in subsequent years, harvest intervals are extended 10- to 20 days beyond the normal 32-to 35-day cutting interval to maximize infestation. Plants are usually scored twice during the growing season, that is, the initial growth and first regrowth (second cutting) in the year of establishment, or the second and third cuttings after the year of establishment.

Individual plants or seeded rows are visually scored for leafhopper yellowing using a 1 to 9 scale (19th Alfalfa Improvement Conference, CR-54-56:63, 1964). A score of 1 = up to 10 percent of leaves show yellowing, 2 = between 10 and 20 percent of leaves yellow, 3 = between 20 and 30 percent

of leaves yellow, 5 = about 50 percent of leaves yellow, and 9 = all leaves yellow. Plants scored 1 to 3 are classified as resistant.

Levels of resistance to leafhopper yellowing among cultivars in a test can be expressed as percentage of resistant plants or as ASI. Resistant and susceptible checks should be included at least once per replication. A single score may be sufficient with a severe infestation of nymphs *(48)*. Infestation levels can be compared among studies when data are obtained as nymphs per gram of dry matter *(45)*.

**Root-Knot Nematode Resistance**

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*Methods used at the Irrigated Agriculture Research and Extension Center, Prosser, Wash.*—The most satisfactory method for evaluating resistance to root-knot nematodes (*Meloidogyne hapla* Chitwood, *M. incognita* Chitwood, and *M. javanica* (Treub) Chitwood) uses nematode larvae applied in an aqueous suspension *(34, 75)*. Evaluations should be conducted in either a greenhouse or a growth chamber. Optimum temperature for tests with *M. hapla* is 25°C *(33)*; optimum temperature for tests with *M. incognita* and *M. javanica* is 30°. A 12- to 16-h photoperiod is recommended for tests with all three nematode species. Seeds of test cultivars are planted in rows in flats filled with steamed or fumigated soil. A spacing of 3 cm between rows with seeds planted 1 to 2 cm apart within rows is optimum. A minimum of 100 plants per cultivar is recommended, divided over at least four replications. Resistant and susceptible check cultivars are included in each test.

Two weeks after planting, an aqueous suspension of active root-knot nematodes is distributed over each flat at the rate of 600 nematodes per seedling *(18)*. Twelve weeks later, the plants are carefully dug and their roots washed and examined for root galls. Each plant is rated on a scale of 1 to 4: 1 = no galls, 2 = 1 to 10 galls, 3 = 10 to 100 galls, and 4 = >100 galls. Care must be used when digging the plants to avoid excess root breakage and loss of galls.

Root-knot nematode resistance among cultivars can be expressed as percentage resistant plants or as ASI. Only class 1 plants are considered resistant when calculating percentage resistant plants. Any galling is considered a susceptible reaction. Cultivar reations are compared by statistical analyses. *M. hapla* resistance ratings for 179 cultivars were reported by Elgin and others *(19)*.

Nematodes for evaluating and screening studies are cultured on roots of tomato plants in pots in the greenhouse (other susceptible hosts also may be used). Nematodes are extracted from the tomato roots by discarding the tops, washing the infected roots in water until free of all soil, and disinfecting the root surface in 10 percent chlorox for 5 min. The roots are placed on a 20-mesh sieve screen, the screen is set in a pan of water, and the water level is raised slightly above the screen surface. Newly hatched nematode larvae begin emerging in less than 24 h, settle into the water below the screen, and continue emerging for 7 to 21 days (or longer). The water is changed daily and the nematodes are stored in a refrigerator at 3°C until they are used. Because nematodes settle in standing water, they can be concentrated by siphoning off excess water. Nematode concentration can be determined by counting the nematodes in several 1-ml samples, using a stereomicroscope. Nematodes should be used as soon as possible after extraction to avoid lowering their infectivity.

A less precise, yet often useful, way of inoculating with root-knot nematodes is to shred infected tomato roots and mix them with the potting soil *(5, 44,* and *82)*. Seeds of test entries are then planted in the soil. A criticism of this method is that hot spots of inoculum often occur, and seedling infection may not be uniform. For preliminary work or general cultivar comparisons, however, this method of inoculation may be more appealing than extracting the nemtodes.

**Stem Nematode Resistance**

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*Methods used at Irrigated Agriculture Research and Extension Center, Prosser, Wash., and University of Nevada, Reno.*—Smith *(80)* was the first scientist to compare levels of stem nematode (*Ditylenchus dipsaci* (Kuhn) Filipjev) resistance in several alfalfa cultivars. Other reports about screening have been published since *(6, 16, 31, 32, 35, 38, 43, 79,* and *90)*. Resistance ratings for 179 cultivars were reported by Elgin and others *(19)*. Generally, techniques that use the degree of cotyledonary node swelling of 7- to 24-day-old seedlings as a resistance indicator have produced variable results and are not recommended. More reliable evaluation techniques delay inoculation until seedlings are about 2 weeks old with resistance ratings made after 16 weeks *(17)*.

Evaluation tests can be conducted either in a greenhouse or growth chamber. A 20°C temperature and 12- to 16-h photoperiod are recommended. Seeds of test cultivars are germinated on filter paper. When seedling radicals are approximately 5 mm long, the seedlings are planted in rows in metal flats filled with steamed or fumigated soil. A fine or very fine sandy loam soil is preferred. Soil mixes containing high levels of peat moss should be avoided. Seedlings are spaced no closer than 2.5 cm apart in rows, with rows no closer than 3 cm. Seedlings then are covered with about

1.0 cm of moist soil and watered. Direct seeding can be used; however, less uniform stands often result.

Plant stands are counted 2 weeks after emergence, and a fine sprinkler can or mist atomizer is used to sprinkle or spray inoculate an aqueous suspension of stem nematodes onto the seedlings. A minimum inoculation rate of 100 nematodes per seedling can be used, but a heavier rate of 200 nematodes per seedlings is recommended. Best results are obtained when the plants and soil are prevented from drying rapidly after inoculation. This can be accomplished by either scheduling greenhouse inoculations late in the day or by turning growth chamber lights off for 12 h after inoculation. Although these procedures are usually adequate, in areas of low humidity covering the plants with wet cheesecloth and a plastic cover to slow drying may be advantageous. To ensure against escapes, a second inoculation with 200 nematodes per seedling should be made 2 weeks after the initial inoculation.

Individual plants are rated 12 weeks after the second inoculation according to swelling and distortion of the lower stems and crown buds caused by stem nematode attack. Plants are rated on a 1 to 5 scale: 1 = no swelling and distortion, 2 = slight swelling but no distinct symptom, 3 = moderate swelling and distortion, 4 = severe swelling and distortion, and 5 = severe swelling, severe necrosis, or death.

The percentage of resistant plants for each cultivar is obtained by considering those plants rated 1 and 2 as resistant, dividing by the number of plants present at the 2-week stand count, and multiplying the quotient by 100 (table 3). Individual plant ratings may also be used to obtain an ASI for each cultivar. A minimum of 100 plants per cultivar is recommended in each test with at least five replications per test. Resistant and susceptible check cultivars are included in each test.

Nematodes for stem nematode studies can be obtained in two ways. The most productive way is to maintain monoxenic cultures of nematodes in the laboratory. This is done by culturing the nematode on alfalfa tissue growing on a nutrient medium *(21)*. Nematodes are extracted from alfalfa tissue by placing the infected plant tissue and medium on a 20-mesh sieve screen covered by two-ply facial tissue. The screen is set in a pan of water so the water barely covers the plant tissue. The nematodes settle in the water below the screen, and the water is collected daily. Most of the nematodes are extracted within 48 h. The nematodes are stored in a refrigerator at 3°C until used. Nematodes will settle in standing water and can be concentrated by siphoning off the upper water. The nematode concentration per milliliter can be determined by counting nematodes in three or four 1-ml samples using a stereomicroscope. Nematodes should be used as soon as possible after extracting to avoid lowering their infectivity.

**Table 3.—Evaluation of 7 alfalfa cultivars for stem nematode resistance 16 weeks after planting and inoculation**

| Cultivar | Stand count, 2 weeks | Plants rated 1 and 2[1] | Resistant plants[2] |
|---|---|---|---|
| | | *Number* | *Percent* |
| Ranger (check) | 98 | 10 | 10.2 |
| Vernal | 94 | 11 | 11.7 |
| Saranac | 98 | 39 | 39.8 |
| DuPuits | 97 | 52 | 53.6 |
| Lahontan (check) | 98 | 60 | 61.2 |
| Washoe | 86 | 51 | 59.3 |
| Apalachee | 96 | 84 | 87.5 |

[1]Plants rated 1 to 5: 1 = no swelling and distortion; 5 = severe swelling, necrosis and distortion, or plant death.

[2]Percentage resistant plants = (number plants rated 1 and 2 divided by 2-week stand count) × 100.

A second way to obtain nematodes is to extract them directly from infected plant material collected from the greenhouse or field using these procedures. Seasonal fluctuations in nematode numbers, however, can cause field collections to be disappointing. Not only is finding large numbers of *D. dipsaci* in the field sometimes difficult, but also contamination with other types of nematodes may occur. Field symptoms are most apparent in early spring when new growth begins and again in the fall following the last cutting. Consequently, one can expect to have the greatest success in collecting large numbers of nematodes in the field during those periods.

## Future Developments

The National Alfalfa Improvement Conference (NAIC) plans to continue monitoring changes in information on the major alfalfa pests in North America. As they occur, descriptions of new pests and new or improved evaluation techniques and check cultivars will be presented in each biennial report of the NAIC. A revision of this publication will be considered after significant changes in alfalfa-pest resistance and research occur.

## Literature Cited


(1) App, B. A., and G. R. Manglitz.
1972. Insects and related pests, ch. 24. *In* C. H. Hanson, ed., Alfalfa science and technology. Agronomy 15:527-554. American Society of Agronomy, Madison, Wis.

(2) Barnes, D. K., C. H. Hanson, F. I. Frosheiser, and others.
1971. Recurrent selection for bacterial wilt resistance in alfalfa. Crop Science 11:545-546.

(3) ______ C. H. Hanson, R. H. Ratcliffe, and others.
1970. The development and performance of Team alfalfa: A multiple pest resistant alfalfa with moderate resistance to the alfalfa weevil. U.S. Department of Agriculture, Agricultural Research Service, ARS 34-115, 41 p.

(4) ______ and R. H. Ratcliffe.
1969. Evaluation of annual species of *Medicago* as sources of alfalfa weevil resistance. Crop Science 9:640-642.

(5) Barrons, K. C.
1938. A method of determining root knot resistance in beans and cowpeas in the seedling stage. Journal of Agricultural Research 57:363-370.

(6) Bingefors, S.
1961. Stem nematode in lucerne in Sweden. II. Resistance in lucerne against stem nematode. Uppsala Lantbrukshoegskolans Annaler 27:385-398.

(7) Bromfield, K. R., and C. G. Schmitt.
1967. Cryogenic storage of conidia of *Peronospora tabacina*. Phytopathology 57:1133.

(8) Browder, L. E.
1971. Pathogenic specialization in cereal rust fungi, especially *Puccinia recondita* f. sp. *tritici:* Concepts, methods of study and application. U.S. Department of Agriculture Technical Bulletin No. 1432, p. 26.

(9) Campbell, T. A., S. A. Ostazeski, and C. H. Hanson.
1969. Dry inoculum for inoculating alfalfa with *Colletotrichum trifolii*. Crop Science 9:845-846.

(10) Carnahan, H. L., R. N. Peaden, F. V. Lieberman, and others.
1963. Differential reactions of alfalfa varieties and selections to the pea aphid. Crop Science 3:219-222.

(11) Cope, W. A.
1957. Inheritance of rust resistance in alfalfa. Dissertation Abstracts 17:481-482.

(12) Cormack, W. M., R. W. Peake, and R. K. Downey.
1957. Studies on methods and materials for testing alfalfa for resistance to bacterial wilt. Canadian Journal of Plant Science 37:1-11.

(13) Devine, T. E., C. H. Hanson, S. A. Ostazeski, and others.
1971. Selection for resistance to anthracnose ("Colletotrichum trifolii") in four alfalfa populations. Crop Science 11:854-855.

(14) ______ R. H. Ratcliffe, C. M. Rincker, and others.
1975. Registration of Arc alfalfa. Crop Science 15:97.

(15) Dudley, J. W.
1963. Registration of Cherokee alfalfa. Crop Science 3:458-459.

(16) Elgin, J. H., Jr., D. W. Evans, and L. R. Faulkner.
1975a. Factors affecting the infection of alfalfa seedlings by *Ditylenchus dipsaci*. Journal of Nematology 7:380-383.

(17) ______ D. W. Evans, and L. R. Faulkner.
1975b. Evaluation of alfalfa for stem-nematode resistance. Crop Science 15:275-276.

(18) ______ F. A. Gray, R. N. Peaden, and others.
1973. Optimum inoculum levels for screening alfalfa seedlings for resistance to northern root-knot nematode in a controlled environment. Plant Disease Reporter 57:657-660.

(19) ______ B. J. Hartman, D. W. Evans, and others.
1980. Stem nematode and northern root-knot nematode resistance ratings for alfalfa cultivars and experimental lines. U.S. Department of Agriculture, Agricultural Research Service, ARR-NE-7, 14 p.

(20) Elling, L. J., and F. I. Frosheiser.
1960. Reaction of twenty-two alfalfa varieties to bacterial wilt. Agronomy Journal 52:241-242.

(21) Faulkner, L. R., D. B. Bower, D. W. Evans, and others.
1974. Mass culturing of *Ditylenchus dipsaci* to yield large quantities of inoculum. Journal of Nematology 6:126-129.

(22) Fried, P. M., and D. L. Stuteville.
1975. Effect of chlorine on *Peronospora trifoliorum* sporangial production and germination. Phytopathology 65:929-930.

(23) ______ and D. L. Stuteville.
1977. *Peronospora trifoliorum* sporangium development and effects of humidity and light on discharge and germination. Phytopathology 67:890-894.

(24) Frosheiser, F. I., and D. K. Barnes.
1973. Field and greenhouse selection for *Phytophthora* root rot resistance in alfalfa. Crop Science 13:735-738.

(25) ______ and D. K. Barnes.
1978. Field reaction of artificially inoculated alfalfa populations to the *Fusarium* and bacterial wilt pathogens alone and in combination. Phytopathology 68:943-946.

(26) Graham, J. H., T. E. Devine, J. E. McMurtrey, and others.
1975. Agar plate method for selecting alfalfa for resistance to *Colletotrichum trifolii*. Plant Disease Reporter 59:382-384.

(27) _____ F. I. Frosheiser, D. L. Stuteville, and others.
1978. A compendium of alfalfa diseases. American Phytopathological Society, St. Paul, Minn., 65 p.

(28) _____ R. R. Hill, Jr., D. K. Barnes, and others.
1965. Effects of three cycles of selection for resistance to common leafspot in alfalfa. Crop Science 5:171-173.

(29) _____ K. W. Kreitlow, and L. R. Faulkner.
1972. Diseases, ch. 23. *In* C. H. Hanson, ed., Alfalfa science and technology. Agronomy 15:497-526. American Society of Agronomy, Madison, Wis.

(30) Gray, F. A., R. B. Hine, M. H. Schonhorst, and others.
1973. A screening technique useful in selecting for resistance in alfalfa to *Phytophthora megasperma.* Phytopathology 63:1185-1188.

(31) Griffin, G. D.
1967. Evaluation of several techniques for screening alfalfa for resistance to *Ditylenchus dipsaci* Plant Disease Reporter 51:651-654.

(32) _____
1968. the pathogenicity of *Ditylenchus dipsaci* to alfalfa and the relationship of temperature to plant infection and susceptibility. Phytopathology 58:929-932.

(33) _____
1969. Effects of temperature on *Meloidogyne hapla* in alfalfa. Phytopathology 59:599-602.

(34) _____ and J. H. Elgin, Jr.
1977. Penetration and development of *Meloidogyne hapla* in resistant and susceptible alfalfa under differing temperatures. Journal of Nematology 9:51-56.

(35) Grundbacher, F. J.
1962. Testing alfalfa seedlings for resistance to the stem nematode *Ditylenchus dipsaci* (Kuhn) Filipjev. Proceedings of the Helminthological Society 29:152-158.

(36) Hackerott, H. L., T. L. Harvey, E. L. Sorensen, and others.
1958. Varietal differences in survival of alfalfa seedlings infested with spotted alfalfa aphids. Agronomy Journal 50:139-141.

(37) _____ E. L. Sorensen, T. L. Harvey, and others.
1963. Reactions of alfalfa varieties to pea aphids in the field and greenhouse. Crop Science 3:298-301.

(38) Hanna, M. R., and E. J. Hawn.
1964. Seedling inoculation studies with the alfalfa stem nematode. Canadian Journal Plant Science 45:357-363.

(39) Hill, R. R., Jr., R. T. Sherwood, and J. W. Dudley.
1963. Effect of recurrent phenotypic selection on resistance of alfalfa to two physiological races of *Uromyces striatus medicaginis.* Phytopathology 53:432-435.

(40) Howe, W. L., and G. R. Pesho.
1960a. Influence of plant age on the survival of alfalfa varieties differing in resistance to the spotted alfalfa aphid. Journal of Economic Entomology 53:142-144.

(41) _____ and G. R. Pesho.
1960b. Spotted alfalfa aphid resistance in mature growth of alfalfa varieties. Journal of Economic Entomology 53:234-238.

(42) Howell, R. K., T. E. Devine, and C. H. Hanson.
1971. Resistance of selected alfalfa strains to ozone. Crop Science 11:114-115.

(43) Hunt, O. J., L. R. Faulkner, and R. N. Peaden.
1972. Breeding for nematode resistance, ch. 16. *In* C. H. Hanson, ed., Alfalfa science and technology. Agronomy 15:355-370. American Society Agronomy, Madison, Wis.

(44) _____ R. N. Peaden, G. D. Griffin, and others.
1969. Development of resistance to root-knot nematode (*Meloidogyne hapla* Chitwood) in alfalfa (*Medicago sativa* L.). Crop Science 9:624-627.

(45) Jarvis, J. L., and W. R. Kehr.
1966. Population counts vs. nymphs per gram of plant material in determining degree of alfalfa resistance to the potato leafhopper. Journal of Economic Entomology 59:427-430.

(46) Kehr, W. R., F. I. Frosheiser, R. D. Wilcoxson, and others.
1972. Breeding for disease resistance, ch. 15. *In* C. H. Hanson, ed., Alfalfa science and technology. Agronomy 15:335-354. American Society Agronomy, Madison, Wis.

(47) _____ and G. R. Manglitz.
1976. Performance of certified seed lots of Dawson alfalfa. Nebraska Agricultural Experiment Station, Research Bulletin No. 277.

(48) _____ R. L. Ogden, and S. D. Kindler.
1970. Diallel analyses of potato leafhopper injury to alfalfa. Crop Science 10:584-586.

(49) _____ R. L. Ogden, and S. D. Kindler.
1975. Management of four alfalfa varieties to control damage from potato leafhopper. Nebraska Agricultural Experiment Station, Research Bulletin No. 275.

(50) Kernkamp, M. F., and Glen Hemerick.
1952. A "deep-freeze" method of maintaining virulent inoculum of the alfalfa wilt bacterium, *Corynebacterium insidiosum.* Phytopathology 42:13.

(51) Kindler, S. D., and W. R. Kehr.
1970. Field tests of alfalfa selected for resistance to potato leafhopper in the greenhouse. Journal of Economic Entomology 63:1464-1467.

(52) Klement, W. J., and N. M. Randolph.
1960. The evaluation of resistance of seedling alfalfa varieties and strains to the spotted alfalfa aphid, *Therioaphis maculata*. Journal of Economic Entomology 53:667-669.

(53) Leath, K. T.
1971. Quality of light required for sporulation by *Leptosphaerulina*. Phytopathology 61:70-72.

(54) ______ and R. R. Hill, Jr.
1974a. Large incubation chamber suited for use in selection for disease resistance. Crop Science 14:901-903.

(55) ______ and R. R. Hill, Jr.
1974b. *Leptosphaerulina briosiana* on alfalfa: Relation of lesion size to leaf age and light intensity. Phytopathology 64:243-245.

(56) Loper, G. M., C. H. Hanson, and J. H. Graham.
1967. Coumestrol content of alfalfa as affected by selection for resistance to foliar diseases. Crop Science 7:189-192.

(57) Miller, P. M.
1955. V-8 juice as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.

(58) Morrison, R. H.
1977. A seedling-box test for evaluating alfalfa for resistance to anthracnose. Plant Disease Reporter 61:35-37.

(59) Newton, R. C., and D. K. Barnes.
1965. Factors affecting resistance of selected alfalfa clones to the potato leafhopper. Journal of Economic Entomology 58:435-439.

(60) Nielson, M. W.
1967. Procedures for screening and testing alfalfa for resistance to the alfalfa seed chalcid. U.S. Department of Agriculture, Agricultural Research Service, ARS 33-120, 10 p.

(61) ______ and H. Don.
1974. A new virulent biotype of the spotted alfalfa aphid in Arizona. Journal of Economic Entomology 67:64-66.

(62) ______ H. Don, M. H. Schonhorst, and others.
1970. Biotypes of the spotted alfalfa aphid in Western United States. Journal of Economic Entomology 63:1822-1825.

(63) ______ and W. F. Lehman.
1977. Multiple aphid resistance in CUF-101 alfalfa. Journal of Economic Entomology 70:13-14.

(64) ______ W. F. Lehman, and R. T. Kodet.
1976. Resistance in alfalfa to *Acyrthosiphon kondoi*. Journal of Economic Entomology 69:471-472.

(65) ______ and M. H. Schonhorst.
1967. Sources of alfalfa seed chalcid resistance in alfalfa. Journal of Economic Entomology 60: 1506-1511.

(66) ______ M. H. Schonhorst, H. Don, and others.
1971. Resistance in alfalfa to four biotypes of the spotted alfalfa aphid. Journal of Economic Entomology 64:506-510.

(67) Ortman, E. E., E. L. Sorensen, R. H. Painter, and others.
1960. Selection and evaluation of pea aphid-resistant alfalfa plants. Journal of Economic Entomology 53:881-887.

(68) Ostazeski, S. A., D. K. Barnes, and C. H. Hanson.
1969. Laboratory selection of alfalfa for resistance to anthracnose, *Colletotrichum trifolii*. Crop Science 9:351-354.

(69) ______ T. A. Campbell, T. E. Devine, and others.
1971. Low temperature maintains virulence of dry *Colletotrichum trifolii* inoculum. Crop Science 11:935.

(70) ______ and J. H. Elgin, Jr.
1980. Physiologic races of *Colletotrichum trifolii*. Phytopathology 70:691.

(71) ______ J. H. Elgin, Jr., and J. E. McMurtrey III.
1979. Occurrence of anthracnose on formerly anthracnose-resistant 'Arc' alfalfa. Plant Disease Reporter 63:734-736.

(72) Painter, R. H.
1951. Insect resistance in crop plants. University Press of Kansas, Lawrence.

(73) Pedersen, M. W., and D. K. Barnes.
1965. Inheritance of downy mildew resistance in alfalfa. Crop Science 5:4-5.

(74) Perkin, D. D.
1962. Preservation of *Neurospora* stock cultures with anhydrous silica gel. Canadian Journal of Microbiology 8:591-594.

(75) Reynolds, H. W., and J. H. O'Bannon.
1960. Reaction of sixteen varieties of alfalfa to two species of root-knot nematodes. Plant Disease Reporter 44:441-443.

(76) Semeniuk, G.
1971. Diseased leaves as a source of ascospores of *Leptotrochila medicaginis* for alfalfa inoculations. Phytopathology 61:910.

(77) ______ and M. D. Rumbaugh.
1976. Reaction of some perennial and annual *Medicago* species and cultivars to the yellow leafblotch disease caused by *Leptotrochila medicaginis*. Plant Disease Reporter 60:596-599.

(78) Shade, R. E., T. E. Thompson, and W. R. Campbell.
1975. An alfalfa weevil larval resistance mechanism detected in *Medicago*. Journal of Economic Entomology 68:399-404.

(79) Sherwood, R. T., J. W. Dudley, T. H. Busbice, and others.
1967. Breeding alfalfa for resistance to the stem nematode *Ditylenchus dipsaci*. Crop Science 7:382-384.

(80) Smith, O. F.
1958. Reactions of some alfalfa varieties to the stem nematode. Phytopathology 48:107.

(81) ______ and R. N. Peaden.
1960. A method of testing alfalfa plants for resistance to the pea aphid. Agronomy Journal 52: 609-610.

(82) Stanford, E. H., B. P. Goplen, and M. W. Allen.
1958. Sources of resistance in alfalfa to the northern root-knot nematode, *Meloidogyne hapla*. Phytopathology 48:347-349.

(83) Stuteville, D. L.
1977. Antibiotics that selectively inhibit bacteria and fungi antagonistic to *Peronospora trifoliorum*. Proceedings of the American Phytopathological Society 4:167-168.

(84) Summers, C. G., and W. F. Lehman.
1976. Evaluation of non-dormant alfalfa cultivars for resistance to the Egyptian alfalfa weevil. Journal of Economic Entomology 69:29-34.

(85) Talboys, P. W.
1960. A culture medium aiding the identification of *Verticillium albo-atrum* and *V. dahliae*. Plant Pathology 9:57-58.

(86) Webster, J. A., E. L. Sorensen, and R. H. Painter.
1960a. Temperature, plant-growth stage, and insect-population effects on seedling survival of resistant and susceptible alfalfa infested with potato leafhoppers. Journal of Economic Entomology 61: 142-145.

(87) ______ E. L. Sorensen, and R. H. Painter.
1968b. Resistance of alfalfa varieties to the potato leafhopper: Seedling survival and field damage after infestation. Crop Science 8:15-17.

(88) Welty, R. E., and H. B. Collins.
1979. Loss of viability of spores of *Colletotrichum trifolii* produced on lima bean agar. Plant Disease Reporter 63:486-489.

(89) ______ and J. P. Mueller.
1979. Occurrence of a highly virulent isolate of *Colletotrichum trifolii* on alfalfa in North Carolina. Plant Disease Reporter 63:666-670.

(90) Wynne, J. C., and T. H. Busbice.
1968. Effects of temperature and incubation period on the expression of resistance to stem nematode in alfalfa. Crop Science 8:179-183.

## Appendix

**Approximate percentages of resistant plants expected for check cultivars used in standard test procedures**

| Disease | | Check cultivars | Approximate resistance expected (%) |
|---|---|---|---|
| Bacterial Wilt | R | Vernal | 42 |
| | S | Narragansett | 1 |
| | S | Sonora | < 1 |
| Anthracnose | R | Arc (race 1) | 75 |
| | S | Arc (race 2) | 0 |
| | R | Saranac AR (race 1) | 65 |
| | R | Saranac AR (race 2) | 55 |
| | S | Saranac (race 1 or race 2) | 0 |
| Common Leaf Spot | R | MSA–CW3An3 | 87 |
| | R | Ramsey | [1]23–70 |
| | S | Ranger | 0 |
| | S | Moapa 69 | 0 |
| Downy Mildew | R | Saranac | [2]20–50 |
| | S | Kanza | 0–2 |
| Fusarium Wilt | R | Agate | 54 |
| | R | Moapa 69 | 76 |
| | S | Narragansett | 22 |
| Lepto Leaf Spot | R | MSA–PL–L | 15–30 |
| | S | Ranger | 0 |
| | S | Moapa 69 | 0 |
| Phytophthora Root Rot | R | Agate | 43 |
| | R | CUF–101 | 25 |
| | R | MnP–D1 | 40 |
| | S | Saranac | 3 |
| | S | Sonora | 2 |
| Rust | R | MSA–CW3An3 | 50 |
| | S | Ranger | 1 |
| | S | Saranac | 1 |
| | S | Moapa 69 | 10 |
| Verticillium Wilt | R | Vertus | 30–50 |
| | S | Vernal | < 7 |
| | S | Saranac | < 5 |
| | S | Sonora | < 2 |
| Yellow Leaf Blotch | R | Teton | 30–60 |
| | S | Ranger | 10–29 |

[1]High levels of resistance found in Minnesota field test, low to moderate levels found in California.

[2]Varies with isolate of causal organism.

| Insect | | Check cultivars | Approximate resistance expected (%) |
|---|---|---|---|
| Alfalfa Seed Chalcid | S | Ranger | 0 |
| | S | Sonora | 0 |
| Alfalfa Weevil | R | Team | [3]100 |
| | R | Arc | 95 |
| | S | Ranger | 200 |
| | S | Saranac | 150 |
| Pea Aphid | R | Kanza | [4]65–75 |
| | R | Baker | 65–75 |
| | R | PA–1 | 690 |
| | R | CUF–101 | 70 |
| | S | Ranger | < 10 |
| | S | Vernal | < 10 |
| | S | Caliverde | < 10 |
| | S | Moapa 69 | < 10 |
| Blue Alfalfa Aphid | R | CUF–101 | [4]70 |
| | S | PA–1 | 25 |
| | S | Caliverde | < 10 |
| Spotted Alfalfa Aphid | R | Kanza | [4]70–80 |
| | R | Baker | 70–80 |
| | R | Mesa–Sirsa | 60–70 |
| | R | CUF–101 | 85 |
| | S | Ranger | < 10 |
| | S | Team | < 10 |
| | S | Caliverde | < 10 |
| Potato Leafhopper Yellowing | R | MSA–CW3An3 | [5]10–30 |
| | S | Ranger | 70–100 |
| **Nematode** | | | |
| Stem Nematode | R | Lahontan | 50–65 |
| | R | Caliverde 65 | 28 |
| | S | Ranger | 8 |
| | S | Moapa 69 | 1–2 |
| Northern Root–Knot Nematode | R | Nev. Syn XX | 92–100 |
| | R | Nev. Syn YY | 91 |
| | S | Lahontan | 3 |
| | R | Mesa–Sirsa | 2 |
| Southern Root–Knot Nematode | R | Moapa 69 | >50 |
| | S | Lahontan | < 5 |
| | S | Caliverde 65 | 10–30 |

[3]For alfalfa weevil, data on percentage of resistant plants not available. Ratings are based on percentage of defoliation of Team (100%).

[4]Data for aphid resistance expressed as percentage of seedlings surviving and includes plants with both high and intermediate levels of resistance.

[5]For potato leafhopper, data on percentage of resistant plants not available. Ratings are based on percent of plant foliage yellowed and correspond to visual yellowing scores of 2–3 for MSA–CW3An3 and 7–9 for Ranger.

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